

REMEDIES FOR CIBOPHOBIA OR LIFESTYLE-RELATED DISEASES AND
METHOD OF SCREENING THE SAME

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Technical Field

The present invention relates to a therapeutic agent for cibophobia, which comprises, as an active ingredient, a substance that suppresses expression or function of orphan GPCR expressed in hypothalamus. Specifically, the present 10 invention relates to a therapeutic agent for cibophobia, which comprises, as an active ingredient, antisense nucleic acid of GPCR mRNA, an expression vector containing the nucleic acid or a host cell transfected with the expression vector, or a therapeutic agent for cibophobia, which comprises, as 15 an active ingredient, a substance having an antagonist activity to GPCR. Furthermore, the present invention relates to a coexpression system of a GPCR and a G protein and a screening method for a therapeutically active compound for cibophobia using the same. The present invention 20 moreover relates to a therapeutic agent for a lifestyle-related disease, which comprises, as an active ingredient, a substance that enhances the expression or function of GPCR. In detail, the present invention relates to a therapeutic agent for a lifestyle-related disease, which 25 comprises, as an active ingredient, a substance having an agonist activity to GPCR. Furthermore, the present invention relates to a coexpression system of a GPCR and a G protein and a screening method for a therapeutically active compound for a lifestyle-related disease using the same.

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Background Art

Due to westernization of the eating habits, increase of social stress and the like in recent years, the number

of patients with obesity and accompanying lifestyle-related diseases, particularly type II diabetes, has been increasing dramatically. For the therapy of these cases, exercise therapy and diet therapy are performed first. When the 5 weight control is insufficient even by these treatments, drug therapy is performed. In doing so, a therapeutic agent which is superior in controlling food intake, body weight and blood glucose, and which is safe has been desired.

On the other hand, stress society of the present age 10 combined with epidemic of an easy diet has brought a rapid increase of psychogenic eating disorder, such as cibophobia, in adolescent women. While it is indispensable to solve psychological problems to treat these diseases fundamentally, drug therapy may be performed to forcibly control feeding 15 behavior as a supportive therapy. A therapeutic agent in this case is also requested to be able to promote eating while controlling body weight and glucose level.

Eating is mainly controlled by the central nervous system, and many nervous systems ruling over instinctive 20 behaviors of human, such as appetite and the like, are located particularly in hypothalamus. In fact, when hypothalamus ventromedial nucleus of rat is damaged, it causes overeating and obesity, whereas when hypothalamus lateral nucleus is damaged, feeding behavior is not taken. In addition, 25 localization of receptors of leptin and neuropeptides (e.g., neuropeptide Y (NPY)), which are involved in eating control, has been shown heretofore, which makes it clear that hypothalamus is an important organ for feeding behavior.

It has become clear that receptors of physiologically 30 active substances in the central nervous system including hypothalamus, particularly G protein-coupled receptor (GPCR), correlates with feeding behavior. For example, it is known that knockout (KO) mouse with serotonin 5-THT_{2C}

receptor suffers from chronic overeating. In addition, melanocortin 4 receptor antagonist increases food intake and, on the contrary, NPY Y5 receptor antagonist suppresses food intake.

5 Thus, stimulation of the nervous system in hypothalamus is considered to influence the feeding behavior, and substitute operation of signal transduction through GPCR, which is expressed in hypothalamus, by the use of a small compound meets the above-mentioned object of controlling
10 food intake, body weight, glucose level and the like. However, a drug having such an action mechanism has not been marketed at present, and development of such a pharmaceutical agent has been highly desired.

It is therefore an object of the present invention to
15 provide a means for regulating feeding behavior by allowing an external factor, particularly a factor that suppresses or promotes expression or function of GPCR involved in the feeding behavior, to function, thereby treating a lifestyle-related disease mainly caused by overeating or
20 obesity, or cibophobia. It is another object of the present invention to provide a compound having a controlling effect on eating disorder such as overeating and apocleisis, obesity or the like, and a screening method for such a compound.

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Disclosure of the Invention

To achieve the above-mentioned objects, the present inventors first examined a gene encoding a receptor expressed in hypothalamus, and, as a result, found a certain orphan GPCR (hereinafter to be referred to as GPRC5D) gene is
30 expressed in hypothalamus of obese model mouse. Next, the present inventors administered an antisense oligo DNA of mRNA encoding this receptor to obese model mouse to inhibit its expression. As a result, food intake was in fact increased

and blood glucose level was elevated. Therefrom it has been clarified that GPRC5D is a receptor involved in the signal transduction which negatively regulates the feeding behavior, and therefore, a substance inhibiting expression or function 5 of this receptor shows a therapeutic effect on eating disorders such as cibophobia. In contrast, a substance enhancing expression or function of this receptor should show a therapeutic effect on lifestyle-related diseases including type II diabetes caused by overeating, obesity and the like.

10 Thus, the present inventors constructed a series of coexpression systems of GPRC5D and various G proteins, and developed a method for screening for a compound having a therapeutic activity against cibophobia or lifestyle-related diseases by searching for an agonist or

15 15 an antagonist to GPRC5D using the system, which resulted in the completion of the present invention.

Accordingly, the present invention provides a therapeutic agent for cibophobia, which comprises, as an active ingredient, a substance that suppresses expression 20 or function of GPRC5D, a GPCR expressed in hypothalamus. As a substance capable of specifically suppressing the expression of GPRC5D, an antisense nucleic acid of mRNA encoding GPRC5D can be preferably mentioned. In this case, the antisense nucleic acid can be provided not only as it 25 is but in the form of an expression vector encoding said nucleic acid or a host cell into which said expression vector has been introduced. In addition, as a substance capable of specifically suppressing the function of GPRC5D, an antagonist to said receptor can be mentioned.

30 The present invention further provides a therapeutic agent for lifestyle-related diseases, which comprises, as an active ingredient, a substance enhancing expression or function of GPRC5D. As a substance capable of specifically

enhancing the function of GPRC5D, a physiological ligand and an agonist to this receptor can be mentioned.

Therefore, another aspect of the present invention provides a screening method for a substance having a therapeutic activity against cibophobia or lifestyle-related diseases, which comprises screening for an antagonist or an agonist to GPRC5D. This method comprises comparing a GDP/GTP exchange reaction of a G protein or the activity of an effector the G protein acts upon, in the presence and absence of the test substance, in a series of receptor - G protein coexpression systems obtained by constructing a constitution unit for a receptor-binding region of each family of G α , wherein one constitution unit comprises a system comprising, as essential elements, at least a lipid bilayer membrane comprising GPRC5D or an equivalent thereof, and a polypeptide comprising at least a receptor-binding region of a G protein α subunit (hereinafter to be also referred to as G α) belonging to a certain family and a guanine nucleotide-binding region of any G protein α subunit.

Accordingly, the present invention also provides a screening system for a substance having a therapeutic activity against cibophobia or a lifestyle-related disease, which comprises the above-mentioned series of receptor-G protein coexpression systems.

The present invention moreover provides a therapeutic agent for cibophobia or a therapeutic agent for a lifestyle-related disease, comprising, as an active ingredient, a substance having a therapeutic activity against cibophobia or a lifestyle-related disease, which is obtained by the above-mentioned screening system or screening method.

Further characteristics and advantages of the present

invention will become clear from the disclosure of "Best Mode for Embodying the Invention" below.

Brief Description of the Drawings

- 5 Fig. 1 shows the effect of administration of antisense DNA of GPRC5D on food intake of obese mouse (immediately after administration - 48 hours later), wherein black columns show food consumption of control DNA administration groups, white columns show that of antisense DNA administration groups (average ± standard deviation, control DNA administration groups n=4, antisense DNA administration groups n=4), and * in the Figure indicates the presence of a significant difference between the both groups (p<0.05, Student's t-test).
- 10 Fig. 2 shows the effect of administration of antisense DNA of GPRC5D on blood glucose level of obese mouse (immediately after administration - 48 hours later), wherein black columns show blood glucose level of control DNA administration groups, white columns show that of antisense DNA administration groups (average ± standard deviation, control DNA administration groups n=4, antisense DNA administration groups n=4), and * in the Figure indicates the presence of a significant difference between the both groups (p<0.05, Student's t-test).
- 15 Fig. 3 shows variation in expression of GPRC5D gene in normal mouse and obese mouse in satiation state and fasting state, wherein black columns show ratio of GPRC5D/GAPDH in the satiation state, white columns show that in the fasting state (average ± standard deviation, n=3 in each group).
- 20 Fig. 4 shows cAMP concentrations in extract from CHO-K1 cell in which GPRC5D was transiently expressed alone or on fusing with various Ga proteins, wherein mock shows CHO-K1 cells transfected with pcDNA3.1(+), GPRC5D shows CHO-K1

cells transfected with pCC5D, GPRC5D-Gs shows CHO-K1 cells transfected with pCC5D/His/G α S2, GPRC5D-Gi shows CHO-K1 cells transfected with pCC5D/His/G α i2 and GPRC5D-Gq shows CHO-K1 cells transfected with pCC5D/His/G α 16.

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Best Mode for Embodying the Invention

The present invention provides a therapeutic agent for cibophobia, which comprises as an active ingredient a substance that suppresses the expression or function of 10 GPRC5D, a GPCR expressed in the hypothalamus.

"GPRC5D" is one of human GPCR proteins consisting of the amino acid sequence shown by SEQ ID NO:2, which has been newly discovered by homology search on EST database using a set of human GPCR amino acid sequence categorized into the 15 5th group of metabotropic glutamate receptor-like family (family C) (Brauner-Osborne et al., *Biochim. Biophys. Acta*, 1518(3): 237-48 (2001); registered in GeneBank under accession numbers: AF209923, XM006896 and NM018654).

However, physiological functions, physiological ligands and 20 coupling G protein (α subunit) subtypes, etc. of GPRC5D have remained unclear. The present inventors have independently found that this receptor gene is expressed in hypothalamus of the obese model mouse and conducted further investigations based on this finding. As a result, as described above, the 25 present inventors have identified this protein as a membrane receptor involved in stimulation of the feeding center.

It is known that GPCR homologs corresponding to human GPRC5D exist in other mammals [e.g., homolog mGprc5d (SEQ ID NO:4) which has about 82% identity with human GPRC5D at 30 the amino acid level was discovered in mouse (Brauner-Osborne et al., ibid. registered in GenBank under accession number: AF218809). Hereinafter a simple reference to "GPRC5D" is to be understood as collectively referring to human GPRC5D and

its mouse homolog.]. Accordingly, the therapeutic agent for cibophobia of the present invention is intended for use to eating disorders not only in humans but also in other mammals. Since social environment of the present age is a stressful 5 environment for animals like livestocks and pets, the present invention is useful for the application to the field of veterinary medicine as well.

The therapeutic agent for cibophobia of the present invention comprises, as an active ingredient, a substance 10 that suppresses the expression or function of GPRC5D. The term "expression", as used herein, refers to a state wherein a receptor protein is produced and functionally located on the cell membrane. Accordingly, the "substance that suppresses the expression" may act at any stage, such as at 15 the gene transcription level, post-transcription regulation level, translation-into-protein level, post-translational modification level, membrane transport level and protein folding level. On the other hand, the "substance that suppresses the function" refers to a substance that acts on 20 a receptor once functionally located on the cell membrane, and that does not cause a shift of the equilibration between the active and inactive forms at least toward the active side.

Examples of substances that suppress the expression of GPRC5D include transcriptional suppressors, RNA 25 polymerase inhibitors, RNA-decomposing enzymes, protein synthesis inhibitors, proteases, and protein denaturants; to minimize the adverse effects on other genes and proteins that are expressed in cells, it is important that the substance should be capable of specifically acting on the 30 target molecule. Accordingly, a preferred embodiment of a substance that suppresses the expression of GPRC5D is an antisense nucleic acid of the GPRC5D mRNA (consisting of the base sequence shown by SEQ ID NO:1 (indicates only ORF) or

SEQ ID NO:3) or its initial transcription product. The term "antisense nucleic acid" refers to a nucleic acid that consists of a base sequence capable of hybridizing to target mRNA (initial transcription product) under the physiological conditions of cells that express the target mRNA (initial transcription product), and that is capable of inhibiting the translation into the polypeptide encoded by the target mRNA (initial transcription product) under the hybridized state. The kind of antisense nucleic acid may be DNA or RNA and may be a DNA/RNA chimera. Additionally, because natural form of antisense nucleic acids have their phosphate di-ester linkage decomposed easily by the nuclease present in cells, the antisense nucleic acid of the present invention can also be synthesized using modified nucleotides such as of the thiophosphate type (phosphate bond P=O replaced with P=S) or 2'-O-methyl type, which types are stable to the nuclease. Other important requirements for designing an antisense nucleic acid include increasing the water solubility and cell membrane permeability; these goals can also be achieved by improving the dosage form such as through the use of liposome or microspheres.

The length of the antisense nucleic acid of the present invention is not particularly limited, as long as the antisense nucleic acid is capable of specifically hybridizing to the GPRC5D mRNA or its initial transcription product, and the antisense nucleic acid may be a sequence comprising a sequence of about 15 bases at the shortest in length or complementary to the entire sequence of the mRNA (initial transcription product) at the longest. From the viewpoint of ease of synthesis, antigenicity concern, and other aspects, there may be mentioned, for example, oligonucleotides consisting of preferably about 15 to about 30 bases. When the antisense nucleic acid is an about 25mer

oligo DNA, the base sequence capable of hybridizing to the GPRC5D mRNA under physiological conditions may be any one, as long as it possesses about 80% identity or more, depending on the base composition of the target sequence.

5 The target sequence for the antisense nucleic acid of the present invention is not particularly limited, as long as it is a sequence such that the translation into GPRC5D protein or a functional fragment thereof is inhibited as a result of hybridization with the antisense nucleic acid, and
10 may be the entire sequence or a partial sequence of the GPRC5D mRNA or may be the intron portion of the initial transcription product. However, when using an oligonucleotide as the antisense nucleic acid, it is desirable that the target sequence should be located between the 5'-terminus of the
15 GPRC5D mRNA and the C-terminus of the coding region (the region shown by base numbers 1-1035 in the base sequence shown by SEQ ID NO:1 or base numbers 1-1047 in the base sequence shown by SEQ ID NO:3). Preferably, the target sequence is located on region between the 5'-terminus and the N-terminus
20 side of the coding region, with greatest preference given to a base sequence in the vicinity of the initiation codon (base numbers 148-150 in the base sequence shown by SEQ ID NO:3). Additionally, it is preferable that the target sequence should be selected such that an antisense nucleic
25 acid complementary thereto does not form a secondary structure such as a hairpin structure.

Furthermore, the antisense nucleic acid of the present invention may be capable of not only hybridizing to the GPRC5D mRNA or its initial transcription product to inhibit the
30 translation, but also binding to the GPRC5D gene, a double-stranded DNA, to form a triple-strand (triplex) to inhibit the transcription into mRNA.

Another preferred embodiment of a substance that

suppresses GPRC5D expression is a ribozyme capable of specifically cleaving within the coding region of the GPRC5D mRNA or its initial transcription product (the base sequence shown by base numbers 1-1035 in the base sequence shown by 5 SEQ ID NO:1 or base numbers 148-1047 in the base sequence shown by SEQ ID NO:3) (including the intron portion in case of the initial transcription product). The term "ribozyme" refers to an RNA having an enzyme activity to cleave nucleic acid. Since it has recently been shown that oligo DNA having 10 the base sequence of the enzyme activity site also possesses nucleic acid cleavage activity, the term ribozyme is used herein to include DNA, as long as it possesses sequence-specific nucleic acid cleavage activity. The most widely applicable ribozyme is self-splicing RNA found in 15 infectious RNA of viroids, virusoids, etc.; such ribozymes include the hammerhead type and the hairpin type. The hammerhead type exhibits enzyme activity with about 40 bases, and is capable of specifically cleaving the target mRNA alone by rendering several bases at each end (about 10 bases in 20 total) adjacent to the hammerhead structure complementary to the desired cleavage site of the mRNA. This type of ribozymes is also advantageous in that they do not attack genomic DNA because their substrate is RNA alone. When the GPRC5D mRNA by itself takes a double-stranded structure, it 25 is possible to render the target sequence single-stranded by using a hybrid ribozyme resulting from the linking of an RNA motif from viral nucleic acid that is capable of specifically binding to RNA helicase [*Proc. Natl. Acad. Sci. USA*, 98(10): 5572-5577 (2001)]. Furthermore, when using the 30 ribozyme in the form of an expression vector containing DNA that encodes the ribozyme, the ribozyme may be a hybrid ribozyme resulting from the further linking of a sequence of modified tRNA to promote the transfer to cytoplasm [*Nucleic*

Acids Res., 29(13): 2780-2788 (2001)].

Another embodiment of a substance that suppresses the expression of GPRC5D is a double-stranded oligo RNA that is complementary to a partial sequence in the coding region of 5 the GPRC5D mRNA or its initial transcription product (including the intron portion in the case of the initial transcription product). What is called RNA interference (RNAi), a phenomenon wherein upon intracellular introduction of a short double-stranded RNA, an mRNA complementary to that 10 RNA is decomposed, has long been known to occur in nematodes, insects, plants, and other organisms. Since this phenomena has recently been found to occur in animal cells as well [Nature, 411(6836): 494-498 (2001)], RNAi is drawing attention for its potential as an alternative to ribozyme.

15 The antisense oligonucleotide and ribozyme of the present invention can be prepared by determining the target sequence for the mRNA or its initial transcription product on the basis of the GPRC5D cDNA sequence or genomic DNA sequence, and synthesizing a complementary sequence using 20 a commercially available DNA/RNA autosynthesizer (Applied Biosystems, Beckman, etc.). A double-stranded oligo RNA possessing RNAi activity can be prepared by synthesizing a sense strand and an antisense strand respectively using a DNA/RNA autosynthesizer, denaturing each strand in the 25 appropriate annealing buffer at about 90°C to about 95°C for about 1 minute, and subsequently annealing them at about 30°C to about 70°C for about 1 to about 8 hours. Additionally, a longer double-stranded polynucleotide can be prepared by synthesizing complementary oligonucleotide strands in 30 alternative overlaps, annealing them, and subsequently subjecting them to ligation with ligase.

A preferred embodiment of a substance that suppresses the functional expression of GPRC5D at the

post-translational level is an antibody against GPRC5D or a fragment thereof. This antibody may be a polyclonal antibody or monoclonal antibody, and can be prepared by a well-known immunological technique. Any fragment of the 5 anti-GPRC5D antibody serves for the purpose, as long as it has an antigen-binding site (CDR) for GPRC5D, and is exemplified by Fab, F(ab')₂, ScFv, minibody, etc.

For example, a polyclonal antibody can be obtained by giving GPRC5D protein or a fragment thereof [may be prepared 10 as a complex cross-linked with a carrier protein such as bovine serum albumin or KLH (Keyhole Limpet Hemocyanin), if necessary] as the antigen, along with a commercially available adjuvant (e.g., complete or incomplete Freund's adjuvant), to an animal by subcutaneous or intraperitoneal 15 administration about 2 to 4 times at intervals of 2 to 3 weeks (the antibody titer of serum separated from drawn blood determined by a commonly known antigen-antibody reaction, and its elevation confirmed in advance), collecting whole blood about 3 to about 10 days after final immunization, and 20 purifying the antiserum. Animals to be administered with the antigen include mammals such as rats, mice, rabbits, goat, guinea pigs and hamsters.

A monoclonal antibody can also be prepared by a cell fusion method (e.g., Takeshi Watanabe, *saibouyugouhou no 25 genri to monokuronaru kotai no sakusei*, Akira Taniuchi and Toshitada Takahashi, eds., "monokuronaru kotai to gan - kiso to rinsho-", pp. 2-14, Science Forum Publishing, 1985). For example, a mouse is given this factor, along with a commercially available adjuvant, 2 to 4 times by subcutaneous 30 or intraperitoneal administration, its spleen or lymph node is collected about 3 days after final administration, and leukocytes are separated. These leukocytes are fused with myeloma cells (e.g., NS-1, P3X63Ag8, etc.) to yield a

hybridoma that produces a monoclonal antibody against this factor. The cell fusion may be achieved by the PEG method [*J. Immunol. Methods*, 81(2): 223-228 (1985)] or the voltage pulsation method [*Hybridoma*, 7(6): 627-633 (1988)]. A 5 hybridoma that produces the desired monoclonal antibody can be selected by detecting in the culture supernatant an antibody that specifically binds to an antigen using well-known EIA, RIA, or the like. Cultivation of a hybridoma that produces a monoclonal antibody can be conducted *in vitro*, 10 or *in vivo* in mice or rats, preferably in ascites fluid of mouse, and the resulting antibody can be obtained from a hybridoma culture supernatant or animal ascites fluid, respectively.

However, in view of therapeutic effect and safety in 15 humans, the anti-GPRC5D antibody of the present invention is preferably a chimeric antibody between a human and another animal (e.g., mice etc.), more preferably a humanized antibody. The term "chimeric antibody", as used herein, refers to an antibody having a variable region (V region) 20 from an immunized animal and a constant region (C region) from a human; "humanized antibody" refers to an antibody wherein all regions except CDR have been replaced with a human antibody. A chimeric antibody or a humanized antibody can, for example, be obtained by cutting out a sequence that 25 encodes a V region or CDR from the gene for a mouse monoclonal antibody prepared in the same manner as above, cloning a chimeric gene resulting from fusion with DNA that encodes a C region of an antibody from human myeloma into an appropriate expression vector, and introducing the vector 30 to an appropriate host cell to express the chimeric gene.

Another preferred embodiment of a substance that suppresses the functional expression of GPRC5D at the post-translational level is an oligonucleotide that

specifically binds to GPRC5D and inhibits its functional expression, i.e., aptamer. An aptamer for GPRC5D can, for example, be obtained by the procedure shown below. First, oligonucleotides (e.g., about 60 bases) are randomly synthesized using a DNA/RNA autosynthesizer to obtain a pool of oligonucleotides. Next, an oligonucleotide that binds to the protein of interest, i.e., GPRC5D is separated using an affinity column. The separated oligonucleotide is amplified by PCR and again screened through the aforementioned selection process. By repeating this process in about five cycles or more, an aptamer showing high affinity for GPRC5D can be selected.

A therapeutic agent for cibophobia comprising as an active ingredient a substance that suppresses the expression of GPRC5D is not capable of exhibiting its therapeutic activity unless it is incorporated in cells of the target tissue (i.e., hypothalamus); its active ingredient, nucleic acid or protein molecule, is not easily absorbable in cells and in addition is likely to undergo rapid decomposition in the body. Additionally, because the uptake of these molecules is usually carried out by endocytosis, they are likely to undergo decomposition by lysosome enzyme. Accordingly, it is important to design a drug delivery system (DDS) wherein a substance that suppresses the expression of GPRC5D is delivered to hypothalamic cells in a stable state so as to increase cell membrane permeability and to promote drug release from lysosome/endosome. For example, in the case of an oligo nucleic acid molecule such as an antisense oligonucleotide, it is possible to improve the stability to nuclease, intracellular transfer, and release from lysosome/endosome by chemical modifications such as PNA resulting from the replacement of the phosphate and sugar portions with peptide bonds, and oligonucleotides having a

morphine backbone in place of a phosphate backbone, as well as nucleic acids with their phosphate linkage or sugar portion (2' position, 3' position, etc.) modified as described above; these modified oligo nucleic acids can easily be prepared 5 using a DNA/RNA autosynthesizer.

On the other hand, cell membrane permeability can also be increased by coupling an accessory group such as poly-L-lysine, avidin, cholesterol or phospholipid to an oligonucleotide or antibody molecule.

10 Furthermore, it is also possible to prepare an oligonucleotide or antibody molecule as incorporated in a cationic liposome. By incorporating in a liposome, the active ingredient is protected against decomposition by nuclease and protease, and is incorporated in cells by 15 endocytosis with the cationic surface of the liposome membrane binding to negatively charged molecules on the cell surface. Cationic liposome can, for example, be prepared by mixing a cationic lipid, such as DOTMA, DDAB or DMRIE, and DOPE, a neutral lipid capable of membrane fusion. Because 20 nucleic acid and proteins are polyanionic, they easily form complexes when mixed with cationic liposomes. Additionally, it is possible to achieve cell-specific targeting by inserting in the liposome membrane an antibody or ligand for a cell surface molecule that is expressed specifically in 25 hypothalamus cells. For example, the anti-GPRC5D antibody itself may be inserted in the liposome membrane.

Additionally, to protect the liposome incorporated by endocytosis against decomposition by lysosome enzyme, it is also preferable to use a pH-sensitive liposome (at acidic 30 pH levels, the membrane becomes unstable and its contents are released from endosomal vesicles to cytoplasm before fusion with lysosome) or a liposome fused with Sendai virus wherein the viral RNA has been completely fragmented by

ultraviolet irradiation etc. (the endocytosis pathway avoided by means of the membrane fusion capability of Sendai virus).

The therapeutic agent for cibophobia of the present invention designed in a dosage form as described above can be administered orally or parenterally by dissolving or suspending in an appropriate sterile vehicle. Examples of parenteral administration route include, for example, but are not limited to, systemic administrations such as intravenous, intra-arterial, intramuscular, intraperitoneal and intratracheal administrations, and local administration in the vicinity of the hypothalamus. Preferably, there may be mentioned local administration to the lateral ventricle.

The dosage of the therapeutic agent for cibophobia of the present invention varies depending on the kind of active ingredient, molecule size, administration route, severity of disease, animal species of administration subject, drug acceptability of administration subject, body weight, age, etc., and normally ranges from about 0.0008 to about 2.5 mg/kg, preferably about 0.008 to about 0.025 mg/kg, based on the amount of active ingredient per day for each adult; such a dose may be administered at a time or in divided portions.

When the substance that suppresses the expression of GPRC5D is a nucleic acid molecule like an antisense nucleic acid, ribozyme or aptamer (hereinafter also referred to as effective nucleic acid molecule), the therapeutic agent for cibophobia of the present invention may comprise as an active ingredient an expression vector that encodes the effective nucleic acid molecule. With regard to the expression vector, oligonucleotide or polynucleotide that encodes the aforementioned effective nucleic acid molecule must be functionally linked to a promoter capable of exhibiting

promoter activity in hypothalamus cells of the recipient mammal, or arranged at a position such that the oligonucleotide or polynucleotide is capable of turning to a form functionally linked to the promoter under particular conditions in hypothalamus cells of the administration animal. Any promoter can be used, as long as it is capable of working in hypothalamus cells of the recipient mammal; such promoters include, for example, viral promoters such as the SV40-derived early promoter, cytomegalovirus LTR, Rous sarcoma virus LTR, MoMuLV-derived LTR and adenovirus-derived early promoter, and mammal constitutive protein gene promoters such as the β -actin gene promoter, PGK gene promoter and transferrin gene promoter. The wording "arranged at a position such that the oligonucleotide or polynucleotide is capable of turning to a form functionally linked to a promoter under particular conditions" means that, for example, the promoter and the oligo(poly) nucleotide that encodes an effective nucleic acid molecule are split by two recombinase recognition sequences arranged in the same direction, which are separated by a spacer sequence long enough to prevent expression of the effective nucleic acid molecule from the promoter, such that the spacer sequence is cleaved out in the presence of a recombinase that specifically recognizes the recognition sequence, thereby the polynucleotide that encodes the effective nucleic acid molecule is functionally linked to the promoter, as described in more detail below.

The expression vector of the present invention contains a transcription termination signal, i.e., a terminator region, preferably at downstream of an oligo(poly) nucleotide that encodes the effective nucleic acid molecule. Furthermore, the expression vector of the present invention may further contain selection marker genes

for transformant selection (genes that confer resistance to such drugs as tetracycline, ampicillin, kanamycin, hygromycin and phosphinoslysine, genes that complement auxotrophic mutation, etc.). When the expression vector has 5 a spacer sequence between recombinase recognition sequences as described above, the selection marker gene may also be arranged in the spacer sequence.

The vector used for the expression vector of the present invention is not particularly limited; examples 10 of vectors that are suitable for administration to mammals such as humans include viral vectors such as retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, pox virus, polio virus, Sindbis virus and Sendai virus. Adenovirus is advantageous in a number of features, including 15 extremely high gene introduction efficiency and the capability of being introduced into non-dividing cells. It should be noted, however, that because the incorporation of the introduced gene into the host chromosome is extremely rare, this gene expression is transient and usually only lasts 20 for about 4 weeks. In view of the persistence of the therapeutic effect, it is also preferable to use an adeno-associated virus, which is of relatively high gene introduction efficiency, which can be introduced to non-dividing cells as well, and which can be incorporated 25 into chromosomes via an inverted terminal repeat sequence (ITR).

Effective nucleic acid molecules such as of antisense nucleic acids and ribozyme are in essence foreign substances; their constitutive and excess expression is highly toxic to 30 the host animal introduced with gene and may cause adverse reactions. Accordingly, in a preferred embodiment of the present invention, the expression vector is capable of allowing an effective nucleic acid molecule to express

time-specifically and/or hypothalamus cell-specifically to avoid the adverse effects of the excess expression of the effective nucleic acid molecule at an unwanted time and/or unwanted site. As a first example of such a vector, there
5 may be mentioned a vector containing an oligo(poly)nucleotide that encodes an effective nucleic acid molecule linked functionally to a promoter derived from a gene specifically expressed in hypothalamus cells of the administration animal. There may be mentioned, for example,
10 the native promoter of the GPRC5D gene.

As a second example of the time-specific and hypothalamus-specific expression vector of the present invention, there may be mentioned a vector containing an oligo(poly)nucleotide that encodes an effective nucleic acid
15 molecule functionally linked to an inducible promoter which regulate an expression by an exogenous substance in trans. For example, when using the metallothionein-1 gene promoter as the inducible promoter, the expression of the effective nucleic acid molecule can be induced
20 hypothalamus-specifically at any time by locally administering an inducer such as a heavy metal, e.g., gold, zinc and cadmium, a steroid, e.g., dexamethasone, an alkylating agent, a chelating agent or a cytokine to the hypothalamus at the desired time.

25 Another preferred example of the time-specific and hypothalamus-specific expression vector of the present invention is a vector having the promoter and the oligo(poly)nucleotide that encodes an effective nucleic acid molecule which are split by two recombinase recognition
30 sequences arranged in the same direction, wherein the recombinase recognition sequences are separated by a spacer sequence sufficiently long to prevent the expression of the effective nucleic acid molecule from the promoter. Solely

introducing the vector in hypothalamus cells does not ensure that the promoter directs the transcription of the effective nucleic acid molecule. However, provided that a recombinase that specifically recognizes the recognition sequence is 5 locally administered to the hypothalamus at the desired time, or an expression vector containing a polynucleotide that encodes recombinase is locally administered to express the recombinase in hypothalamus cells, homologous recombination via the recombinase occurs between the recognition 10 sequences; as a result, the spacer sequence is cleaved out and the oligo(poly)nucleotide that encodes the effective nucleic acid molecule is functionally linked to the promoter, resulting in the hypothalamus-specific expression of the effective nucleic acid molecule at the desired time.

15 It is desirable that the recombinase recognition sequence used in the aforementioned vector should be a heterologous recombinase recognition sequence that is not recognized by endogenous recombinase so as to prevent the recombination by the recombinase present in the recipient. 20 It is desirable, therefore, that the recombinase that act on the vector in trans should also be a heterologous recombinase. Preferred examples of such combinations of heterologous recombinase and the recombinase recognition sequence include, but are not limited to, a combination of 25 Escherichia coli bacteriophage P1-derived Cre recombinase and the lox P sequence, and a combination of yeast-derived Flp recombinase and the frt sequence.

Discovered in a bacteriophage, Cre recombinase is known to work in the specific DNA recombination reaction, 30 not only in prokaryotic cells but also in animal cells which are eukaryotic cells and animal viruses. When two lox P sequences are present on the same DNA molecule in the same direction, Cre recombinase cleaves out the DNA sequence

between the sequences to allow them to form a cyclic molecule (cleavage reaction). On the other hand, in cases where two lox P sequences are present on different DNA molecules one of which is cyclic DNA, the cyclic DNA is inserted to the 5 other DNA molecule via the lox P sequence (insertion reaction) [J. Mol. Biol., 150: 467-486 (1981); J. Biol. Chem., 259: 1509-1514 (1984); Proc. Natl. Acad. Sci. USA, 81: 1026-1029 (1984)]. Example cleavage reactions are reported in cultured animal cells [Nucleic Acids Res., 17: 147-161 10 (1989); Gene, 181: 207-212 (1996)], animal viruses [Proc. Natl. Acad. Sci. USA, 85: 5166-5170 (1988); J. Virol., 69: 4600-4606 (1995); Nucleic Acids Res., 23: 3816-3821 (1995)], transgenic mice [Proc. Natl. Acad. Sci. USA, 89: 6232-6236 15 (1992); Proc. Natl. Acad. Sci. USA, 89: 6861-6865 (1992); Cell, 73: 1155-1164 (1993); Science, 265:103-106 (1994)], etc.

As the promoter for the time-specific and hypothalamus-specific expression vector of the present invention, which is based on the interaction of a 20 recombinase/recombinase recognition sequence, there may preferably be used a virus-derived promoter or a mammal constitutive protein gene promoter to ensure the expression at the desired time and site.

Administration of the therapeutic agent for cibophobia 25 of the present invention comprising an expression vector that encodes an effective nucleic acid molecule as an active ingredient is carried out by either the *ex vivo* method, in which nerve cells of the animal to be treated are taken out from the body, cultured, then returned to the body by 30 introduction, and the *in vivo* method, in which the vector is introduced by directly administering it to the recipient's body. In case of the *ex vivo* method, introduction of the vector into the target cell can be carried out by the

microinjection method, calcium phosphate co-precipitation method, PEG method, electroporation method, etc. In case of the *in vivo* method, the viral vector is administered in the form of an injection or the like intravenously,

5 intra-arterially, subcutaneously, intracutaneously, intramuscularly, intraperitoneally or the like.

Alternatively, administering a vector by intravenous injection etc. may pose a problem with the production of a neutralizing antibody against the viral vector; however, it
10 is possible to mitigate the adverse effects of the presence of the antibody by locally injecting the vector in the vicinity of the hypothalamus, where the target cell is present, e.g., in the lateral ventricle (*in situ* method).

Additionally, when using a non-viral vector as the
15 expression vector that encodes an effective nucleic acid molecule, introduction of the expression vector can be carried out by use of a high molecular carrier such as a poly-L-lysine-nucleic acid complex or liposome encapsulation as described above with respect to dosage forms
20 of therapeutic drugs comprising the effective nucleic acid molecule itself as an active ingredient. Alternatively, it is also possible to introduce the vector directly to the target cell using the particle gun method.

When recombinase itself is locally administered as the
25 trans-acting substance in the use of a vector based on recombinase/recombinase recognition sequence interaction, recombinase, for example, may be injected to the hypothalamus on dissolving or suspending in an appropriate sterile vehicle (e.g., artificial cerebrospinal fluid etc.). On the other
30 hand, when a recombinase expression vector is locally administered to the hypothalamus as the trans-acting substance, the recombinase expression vector may be any vector, as long as it possesses an expression cassette having

the recombinase-encoding polynucleotide functionally linked to a promoter capable of exhibiting promoter activity in hypothalamus cells of the administration subject. When the promoter used is a constitutive promoter, it is desirable 5 that the vector administered to the hypothalamus for preventing the expression of recombinase at unwanted times should be a vector that rarely undergoes incorporation in the host cell chromosome, e.g., adenovirus. However, when using an adenovirus vector, the transient expression of 10 recombinase persists for about 4 weeks at most; if the treatment is prolonged, a second or third administration will be necessary. As another approach to expressing a recombinase at the desired time, there may be mentioned the use of an inducible promoter like the metallothionein gene 15 promoter. In this case, viral vectors of high integration efficiency such as retrovirus can be used.

When the substance that suppresses the expression of GPRC5D is a nucleic acid molecule like an antisense nucleic acid, ribozyme or aptamer, the therapeutic agent for 20 cibophobia of the present invention may contain as an active ingredient a host cell containing an expression vector that encodes an effective nucleic acid molecule as described above. As examples of useful host cells, there may be mentioned autologous cells taken out as target cells from the recipient 25 in the aforementioned ex vivo introduction method for an expression vector, nerve cells taken out from allogenic (e.g., stillborn fetuses, brain death patients, etc., in case of humans) or heterologous (non-human mammals such as swine and simian, in case of humans) individuals, or nerve cells 30 obtained by culturing and differentiating such nerve stem cells or ES cells. Because the central nervous system is the organ/tissue where rejection is most unlikely, even heterologous cells can be allowed to take using a small amount

of immunosuppressant in combination.

In another embodiment, it is possible to transform a resident bacterium in the nasal cavity, throat, oral cavity, intestine, or the like of the recipient animal as the host 5 cell, with an expression vector that encodes an effective nucleic acid molecule by a conventional method, and to deliver the thus-obtained transformant to a site of the recipient where the host cell normally occurs. In recent years, a route other than the blood-brain barrier route has been 10 investigated via which a drug is transferred from the nose directly to the cerebrospinal fluid for delivery to the brain; the use of a nasal cavity resident bacterium suffices that objective.

The dosage of the therapeutic agent for cibophobia of 15 the present invention comprising, as an active ingredient, an expression vector that encodes an effective nucleic acid molecule or a host cell harboring the expression vector varies depending on the kind of active ingredient, molecule size, promoter activity, administration route, severity of 20 disease, animal species of administration subject, drug acceptability of administration subject, body weight, age, etc., and is preferably at a level that causes expression of an effective nucleic acid molecule in an amount equivalent to an appropriate dosage of a therapeutic drug comprising 25 the effective nucleic acid molecule itself as an active ingredient in the body of an animal that has received a vector or host cell, and is exemplified by about 2 to about 20 µg/kg, preferably about 5 to about 10 µg/kg based on the amount of vector per day for each adult.

Because GPRC5D is a membrane receptor protein that mediates signal transduction for negatively regulating food consumption, food intake behavior can be suppressed by enhancing the expression of this receptor. Accordingly, the 30

present invention also provides a therapeutic agent for a lifestyle-related disease comprising a substance that enhances the expression of GPRC5D as an active ingredient. In general, the "lifestyle-related disease" is defined as 5 a group of diseases wherein lifestyles such as dietary habits, exercise habits, resting, smoking and drinking are responsible for the onset and progress thereof. The same term, as used herein, specifically refers to "a group of diseases wherein a therapeutic effect can be achieved by 10 adjusting food intake to reduce it," typically exemplified by diabetes, obesity, hyperlipidemia, hyperuricemia, etc. Patients often have two or more of these diseases at a time.

Examples of substances that enhance the expression of GPRC5D include trans-acting factors capable of promoting RNA 15 transcription from the GPRC5D gene, factors capable of promoting splicing or mRNA transfer to cytoplasm, factors that suppress mRNA decomposition, factors capable of promoting binding of ribosome to mRNA, factors that suppress the decomposition of the GPRC5D protein, and factors that 20 promote the transport of the GPRC5D protein to the membrane; as preferred examples of more directly acting specific substances, there may be mentioned the GPRC5D protein or an equivalent thereof, an expression vector containing a nucleic acid that encodes the GPRC5D, or a host cell harboring 25 the expression vector.

The "GPRC5D protein" as used herein is a protein consisting of the amino acid sequence shown by SEQ ID NO:2 or SEQ ID NO:4; "an equivalent of GPRC5D protein" refers to a polypeptide consisting of an amino acid sequence resulting 30 from the substitution, deletion, insertion, addition or modification of 1 or more (preferably 1 to 50, more preferably 1 to 30, still more preferably 1 to 10, and most preferably 1 to 5) amino acids in the amino acid sequence shown by SEQ

ID NO:2 or SEQ ID NO:4, that exhibits a ligand-receptor interaction equivalent to that of a protein consisting of the amino acid sequence shown by SEQ ID NO:2 or SEQ ID NO:4, and that couples with G α to promote the GDP-GTP exchange reaction of the subunit or, being outside the range of this definition, a protein derived from a different mammal, i.e. orthlog, which is encoded by a gene having the same molecular evolution origin as human or mouse GPRC5D gene. Accordingly, the therapeutic agent for a lifestyle-related disease of the present invention is intended for use to treat diabetes, obesity, hyperlipidemia, hyperuricemia, etc., not only in humans or mice but also in other mammals. Because the number of animals suffering from diseases like lifestyle-related diseases, such as obesity due to excess feeding and a lack of exercise, has been increasing with the recent pet animal boom, the remedy of the present invention is very useful in the field of veterinary medicine as well.

The GPRC5D protein or an equivalent thereof can be isolated from a membrane-containing fraction derived from the hypothalamus tissue of humans or mice, or other mammals such as bovine, swine, simian or rat by affinity chromatography with the anti-GPRC5D antibody.

Alternatively, a DNA clone isolated from a cDNA library or genomic library derived from the tissue with the GPRC5D cDNA clone as a probe can be cloned into an appropriate expression vector, introduced to the host cell, expressed, and purified from the membrane-containing fraction of the cell culture by affinity chromatography with the anti-GPRC5D antibody or His-tag, GST-tag or the like. The equivalent may be partially introduced a mutation by an artificial treatment such as site-directed mutagenesis based on the GPRC5D cDNA sequence (the base sequence shown by base numbers 1-1035 in the base sequence shown by SEQ ID NO:1 or base numbers 148-1047

in the base sequence shown by SEQ ID NO:3). Conservative amino acid substitution is well known; those skilled in the art can introduce a mutation as appropriate in the GPRC5D protein, as long as the receptor characteristics of GPRC5D remain unchanged. However, because the ligand binding domain and preferably the extracellular loop to which an inverse agonist is capable of binding, and the N-terminal strand must be conserved to high extents, it is desirable that a mutation should not be introduced in such regions.

A therapeutic agent for a lifestyle-related disease comprising the GPRC5D protein or an equivalent thereof as an active ingredient can be modified to increase its cell membrane permeability by coupling an accessory group such as poly-L-lysine, avidin, cholesterol or phospholipid component as described above with respect to a therapeutic agent for cibophobia comprising the anti-GPRC5D antibody as an active ingredient. Alternatively, this therapeutic agent can also be prepared by encapsulating the GPRC5D protein or an equivalent thereof into a cationic liposome. Because proteins are poly-anionic, the protein easily forms a complex when mixed with a cationic liposome. Additionally, it is also possible to achieve cell-specific targeting by incorporating into the liposome membrane an antibody or ligand for a cell surface molecule specifically expressed in hypothalamus cells. For example, it is also possible to incorporate the anti-GPRC5D antibody (preferably an antibody not having antagonist activity or inverse agonist activity) to the liposome membrane.

A therapeutic agent for a lifestyle-related disease comprising the GPRC5D protein or an equivalent thereof as an active ingredient can be administered orally or parenterally on dissolving or suspending in an appropriate sterile vehicle. Examples of parenteral administration route include, for

example, but are not limited to, systemic administrations such as intravenous, intra-arterial, intramuscular, intraperitoneal and intratracheal administrations, and local administration in the vicinity of the hypothalamus.

5 Preferably, there may be mentioned local administration to the lateral ventricle.

The dosage of the present therapeutic agent for a lifestyle-related disease varies depending on the administration route, severity of disease, animal species
10 of administration subject, drug acceptability of administration subject, body weight, age, etc., and normally ranges from about 0.0008 to about 2.5 mg/kg, preferably about 0.008 to about 0.025 mg/kg based on the amount of active ingredient per day for each adult; such a dose may be
15 administered at a time or in divided portions.

When the substance that enhances the expression of GPRC5D is the GPRC5D protein or an equivalent thereof, the therapeutic agent for a lifestyle-related disease of the present invention may be an expression vector containing a
20 nucleic acid that encodes such a polypeptide, or may be a host cell harboring the expression vector. The expression vector and host cell used here may be identical to those used for the aforementioned cibophobia remedy. Furthermore, regarding the administration route and dosage for these
25 lifestyle-related disease remedies, those exemplified above with respect to cibophobia remedies can be used preferably.

The present invention also provides a therapeutic agent for cibophobia comprising as an active ingredient a substance that suppresses the function of GPRC5D expressed
30 on the cell membrane of hypothalamus cells, or a therapeutic agent for a lifestyle-related disease comprising as an active ingredient a substance that promotes such function. These therapeutic agents can be obtained by screening for

substances that exhibit agonist activity, antagonist activity or inverse agonist activity to GPRC5D. Accordingly, the present invention also provides at a time a screening method for a substance that suppresses or promotes the 5 function of GPRC5D, and a screening system for the same method.

The term "agonist activity", as used herein, refers to a property by which the substance in question specifically binds to the GPRC5D receptor and causes a shift of the 10 equilibration between the active and inactive forms of GPRC5D toward the active side. Accordingly, substances having agonist activity include physiological ligands for GPRC5D, as well as what is called full agonists and partial agonists. The term "antagonist activity" refers to a property by which 15 the substance in question competitively binds to the ligand-binding site of GPRC5D but has no or almost no effect on the equilibration between the active and inactive forms. Accordingly, substances having antagonist activity are understood to be what is called neutral antagonists, and not 20 to include inverse agonists. The term "inverse agonist activity" refers to a property by which the substance in question binds to any site of GPRC5D and causes a shift of the equilibration between the active and inactive forms of GPRC5D toward the inactive side. The simple term "ligand" 25 as used herein, is understood to include all physiological ligands, agonists, antagonists and inverse agonists.

The screening system of the present invention is a series of receptor-G protein co-expression systems obtained by constructing a constituent unit for the receptor-binding 30 region of each G α family (i.e., G α_s , G α_i , G α_q), which constituent unit consists of a lipid bilayer membrane containing the GPRC5D protein or an equivalent thereof, and a polypeptide comprising at least the receptor-binding

region of a $\text{G}\alpha$ belonging to a family and the guanine nucleotide-binding region of any $\text{G}\alpha$, as essential member constituents. The GPRC5D protein or an equivalent thereof is identical to that mentioned above as an active ingredient 5 of the aforementioned therapeutic agent for a lifestyle-related disease. Although the lipid bilayer membrane retaining the GPRC5D protein or an equivalent thereof may be of any origin, as long as the receptor protein is allowed to take the essential steric structure on the 10 membrane, it is preferably exemplified by fractions containing the cell membrane of eukaryotic cells such as human, bovine, swine, simian, mouse, rat or other mammal cells, and insect cells, e.g., intact cells, cell homogenates, or cell membrane fractions fractionated from these homogenates by 15 centrifugation etc.. However, an artificial lipid bilayer membrane prepared by a conventional method from a solution of various lipids, e.g., phosphatidylcholine, phosphatidylserine, and cholesterol, mixed at an appropriate ratio, preferably a ratio close to abundance ratios in the 20 cell membranes of eukaryotic cells such as mammal cells and insect cells, can also be used preferably in an embodiment of the present invention.

$\text{G}\alpha$ ($\text{G}\alpha_s$), belonging to the G_s family, promotes the activity of adenylate cyclase as the effector, and is 25 exemplified by $\text{G}\alpha_{s-1}$ - $\text{G}\alpha_{s-4}$ and G_{olf} . $\text{G}\alpha$ ($\text{G}\alpha_i$), belonging to the G_i family, suppresses the activity of adenylate cyclase as the effector, and is exemplified by $\text{G}\alpha_{i-1}$ - $\text{G}\alpha_{i-3}$ and G_z . $\text{G}\alpha$ ($\text{G}\alpha_q$), belonging to the G_q family, promotes the activity of phospholipase C as the effector, and is exemplified by $\text{G}\alpha_q$ 30 and G_{16} . The $\text{G}\alpha_s$ polypeptide, $\text{G}\alpha_i$ polypeptide and $\text{G}\alpha_q$ polypeptide of the present screening system need to have a region involved in the binding to its own GPCR (RB region) and a region involved in the binding to any guanine nucleotide

of G α (GB region). Results of X-ray crystallographic analysis of G α have shown that GPCR-binding region is located in the vicinity of the C-terminus whereas the GB region is a region homologous to the nucleotide-binding site of the 5 ras protein (from the N-terminus side: amino acid motives called the P box, G' box, G box, and G" box, the leader of the α E helix in a highly helical domain, α F helix, etc.). When a physiological ligand or agonist to GPRC5D binds to the receptor, the G α activation domain of the receptor and 10 the G α RB region that couples with the receptor interact with each other to produce a conformational change in G α , resulting in the dissociation of GDP from the GB region and quick binding of GTP. G α -GTP acts on the effector to promote or suppress its activity. On the other hand, binding an inverse agonist 15 inactivates the G α active domain due to a conformational change in the receptor, resulting in a decreased active G α -GTP level and inhibition of its action on the effector. Here, provided that a GTP analogue that does not undergo hydrolysis by the GTPase activity of G α , such as 35 S-labeled GTPyS, has 20 been added to the system in place of GTP, it is possible to evaluate the effects of the test substance on the GDP-GTP exchange reaction in G α by determining and comparing the radioactivity bound to the membrane in the presence and absence of the test substance, and to screen for substances 25 that possess ligand activity for GPRC5D. Hence, provided that the radioactivity has increased in the presence of a test substance, the test substance can be judged to possess agonist activity to GPRC5D and hence therapeutic activity for lifestyle-related disease. Conversely, provided that 30 the radioactivity has decreased, the test substance can be judged to possess inverse agonist activity to GPRC5D and hence therapeutic activity for cibophobia.

Once ligands for GPRC5D are screened for, a family that

couples with the receptor is elucidated; subsequent screening can be conducted using only a system containing a G α polypeptide belonging to the family as a member constituent. The results described below of constitutive 5 activation of GPRC5D using a receptor-G α fusion protein expression system strongly suggest that the G protein α subunit capable of coupling with GPRC5D may be G α_s . Accordingly, the present invention also provides a screening method for ligands for the receptor characterized in that 10 the GDP-GTP exchange reaction of the G α or the activity of the effector that interacts with the G α is compared in the presence and absence of the test substance in a co-expression system of a G protein α subunit (preferably G α_s) capable of coupling with GPRC5D and the receptor.

15 The activity of a ligand for GPRC5D can also be determined with the action of a G α polypeptide on the effector as an index. In this case, the screening system of the present invention must further comprise as another constituent a lipid bilayer membrane containing the effector, 20 in addition to the GPRC5D protein or an equivalent thereof. Additionally, the G α polypeptide must further comprise a region for interaction with the effector. Because the G α members belonging to the individual families differ with respect to the kind of effector or the direction of action, 25 it is preferable that all G α polypeptides share the effector interaction region, rather than each has its own effector interaction region. Accordingly, in the present screening system, at least two kinds of G α polypeptides are chimeric polypeptides containing the effector interaction region of 30 a G α belonging to another family. For example, when using phospholipase C as the effector, the G α_q polypeptide may be a native one; however, the G α_s polypeptide and G α_i polypeptide must be chimeric polypeptides wherein the effector

interaction region has been replaced with that of $\text{G}\alpha_q$. As the simplest example of a chimeric polypeptide containing the effector interaction region of a $\text{G}\alpha$ belonging to another family, there may be mentioned a chimeric polypeptide wherein 5 about 5 amino acids at the C-terminus of a $\text{G}\alpha$ belonging to another family (i.e., RB region) have been replaced with its own C-terminal sequence.

In the present screening system, when three kinds of 10 $\text{G}\alpha$ polypeptides contain an effector interaction region of $\text{G}\alpha_s$ or $\text{G}\alpha_i$, a lipid bilayer membrane containing adenylyl cyclase is used as the effector. On the other hand, when the 15 $\text{G}\alpha$ polypeptide contains the effector interaction region of $\text{G}\alpha_q$, a lipid bilayer membrane containing phospholipase C must be used as the effector.

15 In a screening system comprising adenylyl cyclase (hereinafter also referred to as AC) as the effector, the action of a $\text{G}\alpha$ polypeptide on the effector can be evaluated by directly determining the AC activity. The AC activity can 20 be determined using any commonly known technique; examples of useful methods include, but are not limited to, a method comprising adding ATP to an AC-containing membrane fraction, and determining the resulting cAMP content by competitive immunoassay using an anti-cAMP antibody in the presence of cAMP labeled with a RI (e.g., ^{125}I), an enzyme (e.g., alkaline 25 phosphatase, peroxidase, etc.), a fluorescent substance (e.g., FITC, rhodamine, etc.), or the like, and a method comprising adding [α - ^{32}P] ATP to an AC-containing membrane, separating the resulting [^{32}P] cAMP using an alumina column etc., and subsequently determining the radioactivity thereof. 30 When the $\text{G}\alpha$ polypeptide contains the effector interaction region of $\text{G}\alpha_s$, and determining and comparing the AC activity in the presence and absence of a test substance, provided that the AC activity has increased in the presence of the

test substance, the test substance can be judged to possess agonist activity to GPRC5D and hence therapeutic activity for lifestyle-related disease. Conversely, provided that the AC activity has decreased, the test substance can be
5 judged to possess inverse agonist activity to GPRC5D and hence therapeutic activity for cibophobia. On the other hand, when the G α polypeptide contains the effector interaction region of G α , provided that the AC activity has increased in the presence of a test substance, the test substance can be judged
10 to possess inverse agonist activity to GPRC5D and hence therapeutic activity for cibophobia. Conversely, provided that the AC activity has decreased, the test substance can be judged to possess agonist activity to GPRC5D and hence therapeutic activity for lifestyle-related disease.

15 When using an intact eukaryotic cell as the screening system, the action of a G α polypeptide on AC can also be evaluated by determining the intracellular cAMP content, or labeling the cell with [3 H] adenine, and determining the radioactivity of resulting [3 H] cAMP. Although the
20 intracellular cAMP content can be determined by incubating cells in the presence and absence of the test substance for an appropriate time, subsequently disrupting the cells, and subjecting the thus-obtained extract by the aforementioned competitive immunoassay, any other commonly known method can
25 be used.

In another embodiment, the cAMP content may be evaluated by determining the amount of expression of reporter gene under the control of the cAMP-response element (CRE). The expression vector used here is described in detail below,
30 and is outlined below. The intracellular cAMP content is determined by culturing a eukaryotic cell incorporating a vector containing an expression cassette with a DNA that encodes the reporter protein linked downstream of a

CRE-containing promoter, in the presence and absence of the test substance for an appropriate time, disrupting the cells, and measuring and comparing the expression of the reporter gene in the thus-obtained extract using a commonly known 5 technique.

Accordingly, when the G α polypeptide contains the effector interaction region of G α_s , provided that the intracellular cAMP content (or the amount of expression of reporter gene under the control of CRE) has increased in the 10 presence of a test substance, the test substance can be judged to possess agonist activity to GPRC5D and hence therapeutic activity for lifestyle-related disease. Conversely, provided that the cAMP content (or the amount of expression of reporter gene) has decreased, the test substance can be 15 judged to possess inverse agonist activity to GPRC5D and hence therapeutic activity for cibophobia. On the other hand, when the G α polypeptide contains the effector interaction region of G α_i , provided that the intracellular cAMP content (or the amount of expression of reporter gene under the control of 20 CRE) has increased in the presence of a test substance, the test substance can be judged to possess inverse agonist activity to GPRC5D and hence therapeutic activity for cibophobia. Conversely, provided that the cAMP content (or the amount of expression of reporter gene) has decreased, 25 the test substance can be judged to possess agonist activity to GPC5D and hence therapeutic activity for lifestyle-related disease.

On the other hand, in a screening system containing phospholipase C (hereinafter also referred to as PLC) as the 30 effector (i.e., a case wherein the G α polypeptide contains the effector interaction region of G α_q), the action of the G α polypeptide on the effector can be evaluated by directly determining the PLC activity. The PLC activity can, for

example, be evaluated by adding ^3H -labeled phosphatidylinositol 4,5-diphosphate to a PLC-containing membrane fraction, and determining the amount of inositol phosphate produced using a commonly known technique. The PLC activity is determined and compared in the presence and absence of the test substance; provided that the PLC activity has increased in the presence of the test substance, the test substance can be judged to possess agonist activity to GPRC5D and hence therapeutic activity for lifestyle-related disease.

Conversely, provided that the PLC activity has decreased, the test substance can be judged to possess inverse agonist activity to GPRC5D and hence therapeutic activity for cibophobia.

When using an intact eukaryotic cell as the screening system, the action of $\text{G}\alpha$ polypeptide on PLC can also be evaluated by adding [^3H]inositol to the cell and determining the radioactivity of the resulting [^3H] inositol phosphate, or determining the intracellular Ca^{2+} content. Although the intracellular Ca^{2+} content can be determined by spectroscopy using a fluorescent probe (fura-2, indo-1, fluo-3, Calcium-Green I, etc.) or by using a calcium-sensitive luminescent protein such as aequorin after the cells are incubated for a given time in the presence and absence of the test substance, any other commonly known method can be used. As an apparatus suitable for spectroscopy using a fluorescent probe, there may be mentioned the FLIPR (Molecular Devices Company) system.

In another embodiment, the Ca^{2+} content may be evaluated by determining the amount of expression of reporter gene under the control of Ca^{2+} -upregulated TPA (12-O-tetradecanoylphorbol-13-acetate)-respondse element (TRE). The expression vector used in that method is described in detail below, and is outlined below. The

intracellular Ca^{2+} content is determined by culturing a eukaryotic cell incorporating a vector containing an expression cassette with a DNA that encodes the reporter protein linked downstream of a TRE-containing promoter, in 5 the presence and absence of the test substance for an appropriate time, disrupting the cells, and measuring and comparing the expression of the reporter gene in the thus-obtained extract using a commonly known technique.

Accordingly, provided that the intracellular Ca^{2+} 10 content (or the amount of expression of reporter gene under the control of TRE) has increased in the presence of a test substance, the test substance can be judged to possess agonist activity to GPRC5D and hence therapeutic activity for lifestyle-related disease. Conversely, provided that the 15 intracellular Ca^{2+} content (or the amount of expression of reporter gene) has decreased, the test substance can be judged to possess inverse agonist activity to GPRC5D and hence therapeutic activity for cibophobia.

The substance subjected to the screening method of the 20 present invention may be any commonly known compound or a new compound, and is exemplified by compound libraries prepared using combinatorial chemistry techniques, random peptide libraries prepared by solid phase synthesis or the phage display method, and naturally occurring components 25 such as those derived from microorganisms, animals, plants, and marine organisms.

A preferred embodiment of a system containing as essential constituents a lipid bilayer membrane containing the GPRC5D protein or an equivalent thereof, and $\text{G}\alpha$ 30 polypeptide, which system is a constitution unit of the screening system of the present invention, is a host eukaryotic cell transfected with both an expression vector containing a DNA that encodes the GPRC5D protein or an

equivalent thereof and an expression vector containing a DNA that encodes a polypeptide at least comprising the RB region of a G α belonging to a family and the GB region of any G α , a homogenate of the cell, or a membrane fraction from the 5 cell.

The "DNA that encodes the GPRC5D protein or an equivalent thereof" is not particularly limited, as long as it is a DNA that encodes a polypeptide consisting of the amino acid sequence shown by SEQ ID NO:2 or SEQ ID NO:4, or a 10 polypeptide that consists of an amino acid sequence resulting from the substitution, deletion, insertion, addition or modification of 1 or more (preferably 1 to 50, more preferably 1 to 30, still more preferably 1 to 10, and most preferably 1 to 5) amino acids in the amino acid sequence shown by SEQ 15 ID NO:2 or SEQ ID NO:4, that exhibits a ligand-receptor interaction equivalent to that of GPRC5D, and that couples with G α to promote the GDP-GTP exchange reaction of the subunit or a DNA that encodes an ortholog of a polypeptide consisting of the amino acid sequence shown by SEQ ID NO:2 20 or SEQ ID NO:4. As such DNAs, there may be mentioned, for example the GPRC5D cDNA coding region (the base sequence shown by base numbers 1-1035 in the base sequence shown by SEQ ID NO:1 or the base sequence shown by base numbers 148-1047 in the base sequence shown by SEQ ID NO:3), as well as DNAs that 25 encodes a GPCR corresponding to GPRC5D of non-human or mouse mammal origin such as of bovine, swine, simian, or rat; these can be isolated from cDNA libraries or genomic libraries derived from cerebral nerve tissue, including the mammal hypothalamus using the GPRC5D cDNA as a probe. The 30 equivalent may partially incorporate a mutation introduced by an artificial treatment such as site-directed mutagenesis based on the GPRC5D cDNA.

The DNAs that encode the three kinds of G α polypeptides

needs to have at least a sequence that encodes the RB region of the G α in each family, and a sequence that encodes the GB region of any G α . The sequences of the various G α genes are commonly known and the RB region and GB region are well known from the results of X-ray crystallographic analysis of G α as described above. Accordingly, those skilled in the art can easily construct a fragment lacking a portion of the coding sequence of G α as desired.

In a screening system based on the action of G α polypeptide on the effector as an index, the DNA that encodes the G α polypeptide must further contain a nucleic acid sequence that encodes the effector interaction region. Because the three kinds of G α polypeptides share the effector interaction region as described above, at least two kinds of G α polypeptides are chimeras having the effector interaction region of different families. As the simplest example of a DNA that encodes the chimeric polypeptide, there may be mentioned a chimeric polypeptide wherein cDNA sequence encoding about 5 amino acids at the C-terminus of a G α containing the desired effector interaction region have been replaced with a DNA sequence that encodes the C-terminal sequence of a G α belonging to another family.

The DNA that encodes the GPRC5D protein or an equivalent thereof and the DNA that encodes the G α polypeptide must be functionally linked to a promoter capable of exhibiting promoter activity in the host eukaryotic cell. Any promoter can be used, as long as it is capable of working in eukaryotic cell; such promoters include, for example, viral promoters such as the SV40-derived early promoter, cytomegalovirus LTR, Rous sarcoma virus LTR, MoMuLV-derived LTR and adenovirus-derived early promoter, and eukaryote-derived constitutive protein gene promoters such as the β -actin gene promoter, PGK gene promoter and

transferrin gene promoter. It is preferable that the expression vector used contain in addition to the aforementioned promoter a transcription termination signal, i.e., a terminator region, downstream thereof, and it is 5 desirable that the expression vector has an appropriate restriction endonuclease recognition site, preferably a unique restriction endonuclease recognition site that cleaves the vector only at one position, so that a coding DNA can be inserted between the promoter region and the 10 terminator region. Furthermore, the expression vector may further contain a selection marker gene (drug resistance genes such as for tetracycline, ampicillin, kanamycin, hygromycin and phosphinothricin, auxotrophic mutation complementary genes, etc.).

15 As examples of vectors useful in the screening system of the present invention, there may be mentioned plasmid vectors, viral vectors that are suitable for use in mammals such as humans, including retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, pox 20 virus, polio virus, Sindbis virus and Sendai virus, and vaculovirus vectors that are suitable for use in insect cells.

The DNA that encodes the GPRC5D protein or an equivalent thereof and the DNA that encodes the $\text{G}\alpha$ polypeptide may be co-transfected to the host cell as carried on two 25 separate expression vectors, or introduced to the host cell as inserted in a single vector dicistrionically or monocistrionically.

The host cell may be any one, as long as it is a eukaryotic cell such as a mammal cell such as a human, simian, 30 mouse, rat or hamster cell, or an insect cell. Specifically, such host cells include mouse-derived cells such as COP, L, C127, Sp2/0, NS-1, NIH3T3 and ST2, rat-derived cells, hamster-derived cells such as BHK and CHO, simian-derived

cells such as COS1, COS3, COS7, CV1 and Vero, and human-derived cells such as HeLa and 293, as well as insect-derived cells such as Sf9, Sf21 and High Five.

Gene introduction to the host cell can be achieved 5 using any commonly known method applicable to gene introduction to eukaryotic cells; examples of such methods include the calcium phosphate co-precipitation method, the electroporation method, the liposome method, and the microinjection method.

10 The gene-incorporating host cell can, for example, be cultured using a minimum essential medium (MEM) containing about 5% to about 20% bovine fetal serum, Dulbecco's modified Eagle medium (DMEM), RPMI1640 medium, 199 medium, etc. Medium pH is preferably about 6 to about 8; culturing 15 temperature is normally about 27°C to about 40°C.

The thus-obtained eukaryotic cell incorporating a DNA that encodes the GPRC5D protein or an equivalent thereof and a DNA that encodes a G α polypeptide may be used as an intact cell as is, depending on the screening method used, or may 20 be used in the form of a cell homogenate obtained by disrupting the cell in an appropriate buffer solution, or a membrane fraction isolated by centrifuging the homogenate under appropriate conditions (e.g., supernatant recovered via centrifugation at about 1,000 $\times g$, followed by centrifugation 25 at about 100,000 $\times g$ and recovery of the precipitation).

For example, when the ligand characteristics of the test substance are evaluated by GTP γ S-binding assay or direct determination of the effector activity, the screening system used is preferably a membrane fraction prepared from cells 30 as described above. On the other hand, when the ligand characteristics of the test substance are evaluated by determining the intracellular cAMP content (or the amount of expression of cAMP-response reporter) or intracellular

Ca^{2+} content (or the amount of expression of Ca^{2+} -response reporter), the screening system used is an intact eukaryotic cell.

When evaluating ligand activity with the amount of expression of a cAMP-responding reporter (in cases where the effector is adenylate cyclase) or Ca^{2+} -responding reporter (in cases where the effector is phospholipase C) as an index, the host eukaryotic cell must incorporate a vector containing an expression cassette wherein a DNA that encodes the reporter protein is functionally linked downstream of a promoter region containing a cAMP-responding element (CRE) or TPA-responding element (TRE). CRE is a cis-element that activates gene transcription in the presence of cAMP, exemplified by a sequence containing TGACGTCA as a consensus sequence, and may be a sequence containing a deletion, substitution, insertion or addition, as long as cAMP responsiveness is retained. On the other hand, TRE is a cis-element that activates gene transcription in the presence of Ca^{2+} , exemplified by a sequence containing TGACTCA as a consensus sequence, and may be a sequence containing a deletion, substitution, insertion or addition, as long as Ca^{2+} responsiveness is retained. As a CRE- or TRE-containing promoter sequence, there may be used in the same manner virus promoters and eukaryotic cell constitutive protein gene promoters as described above; using a restriction enzyme and DNA ligase, or by means of PCR etc., the CRE or TRE sequence can be inserted downstream of the promoter sequence. As the reporter gene under the control of CRE or TRE, there may be used any commonly known gene that permits quick and simple detection and quantitation of the expression thereof; such genes include, for example, but are not limited to, DNAs that encode such reporter proteins as luciferase, β -galactosidase, β -glucuronidase, alkaline phosphatase and peroxidase. More

preferably, a terminator sequence is arranged downstream of the reporter gene. As such a vector carrying a CRE (or TRE)-reporter expression cassette, there may be used a commonly known plasmid vector or viral vector.

5 Another preferred example of a system containing as essential constituents a lipid bilayer membrane containing the GPRC5D protein or an equivalent thereof, and a G α polypeptide, which system is a constitution unit of the screening system of the present invention, is a host
10 eukaryotic cell transfected with an expression vector containing a DNA that encodes a fused protein wherein a polypeptide at least comprising the RB region of a G α belonging to a family and the GB region of any G α is linked to the C-terminus side of the GPRC5D protein or an equivalent
15 thereof, a homogenate of the cell, or a membrane fraction from the cell.

A DNA encoding GPRC5D protein or an equivalent thereof, and a DNA encoding a polypeptide containing an RB-binding region of G α of each family and a GB region of any G α can
20 be obtained as mentioned above. Those of ordinary skilled in the art can easily construct a DNA encoding a fused protein of GPRC5D and G α polypeptide by appropriately combining known genetic engineering methods based on these DNA sequences. For example, a method comprising ligating a DNA encoding
25 GPRC5D, whose termination codon has been deleted, with a DNA encoding GPRC5D to match reading frame using PCR and the like can be mentioned. In this case, deleting a part of C-terminal of GPRC5D and inserting a linker sequence such as His-tag between GPRC5D and G α are also possible.

30 A DNA encoding the obtained fused protein is inserted into an expression vector as mentioned above, and introduced into an eucaryotic host cell by the above-mentioned gene introduction technique. When the fused protein is expressed

on the obtained eucaryotic cell membrane, and when GPRC5D and G α can interact, G α active domain on intracellular loop 3 of the receptor and RB region of G α interact in the absence of a physiological ligand for the receptor, and can promote 5 the GDP/GTP exchange reaction in G α . In other words, G α stays constitutively being activated. In contrast, a fused protein with G α that does not interact with GPRC5D does not activate GPRC5D, and does not increase G α -GTP level. Here, 10 when a GTP analog free of hydrolysis by GTPase activity of G α , such as 35 S-labeled GTP γ S, is added to the system instead of GTP, activation of GPRC5D can be evaluated by measuring the radioactivity bound with the membrane and comparing with each other in the membrane systems respectively containing 15 three kinds of fused proteins, thereby identifying the G α capable of interacting with the receptor.

Once a G α capable of interacting with GPRC5D is identified, the subsequent screening can be conducted using only a membrane system containing GPRC5D and the G α , preferably only a membrane system containing a fused protein 20 of GPRC5D and G α . In other words, in the same manner as in the identification of the above-mentioned coupled G α , the effect of a test substance on the GDP/GTP exchange reaction in G α can be evaluated by adding, to the system, a GTP analog 25 free of hydrolysis due to GTPase activity of G α , and measuring and comparing the radioactivities bound with the membrane in the presence and absence of the test substance, and a substance having a GPCR ligand activity can be screened for. When the radioactivity increases in the presence of a test substance, the test substance has an agonist activity to 30 GPRC5D, and when the radioactivity decreases, the test substance has an inverse agonist activity to GPRC5D. Since a receptor is activated only partially by a fused protein, when a physiological ligand or an agonist to GPRC5D is bound,

the activity-non-activity balance of the receptor shifts toward the active side, and the G α -GTP level increases further. Thus, this screening system can screen for agonists as well.

As shown in the Examples below, because GPRC5D is 5 constitutively activated only when expressed as a fused protein with G α_s , G α coupled with the receptor is strongly suggested to be G α_s . Therefore, the present invention also provides a screening method for a ligand for the receptor, which comprises comparing, in a fused protein expression 10 system of G α_s and the receptor, a GDP/GTP exchange reaction of G α_s in the presence and absence of the test substance.

Activation of GPRC5D in a fused protein can be also evaluated using, as an index, an action of G α on an effector. In this case, the screening system of the present invention 15 needs to be a membrane system encompassing, in addition to each fused protein, a lipid bilayer further containing an effector each G α interacts with. That is, a membrane system containing a fused protein with G α_q further contains phospholipase C (PLC), a membrane system containing a fused 20 protein with G α_i and G α_s further contains adenylate cyclase (AC). In this case, the presence or absence of activation of GPRC5D can be also evaluated by preparing, for each G α , a membrane system containing GPRC5D and G α separately (that 25 is, not as a fused protein), and measuring and comparing the activity of effector (that is, in a membrane system containing a fused protein of GPRC5D and G α capable of interaction, the activity of effector is significantly high (low in the case of G α_i) as compared to a membrane system containing the both as non-fused proteins, and for those that do not interact, 30 there is no significant difference in the activity of effector between the both systems).

Once a G α capable of interacting with GPRC5D is identified, the subsequent screening can be conducted using

only a membrane system containing a fused protein with said G α by directly or indirectly measuring and comparing the activity of an effector the G α can interact with, in the presence and absence of the test substance. Therefore, the 5 present invention also provides a screening method for a ligand for GPRC5D, which comprises measuring and comparing, in a membrane system containing a fused protein of G α capable of being coupled with a receptor identified by the above-mentioned identification method of G α coupled with 10 GPRC5D and the receptor and an effector with which said G α is capable of interaction, the activity of an effector, in the presence and absence of the test substance.

As mentioned above, because GPRC5D is constitutively activated only when expressed as a fused protein with G α_s , 15 G α coupled with the receptor is strongly suggested to be G α_s . Therefore, the AC activity in a membrane system containing a fused protein of GPRC5D and G α_s is measured and compared in the presence and absence of the test substance. The AC activity can be measured in the same manner as in the above.

It is also known that G α can be constitutively activated by introducing a mutation by a known method into a specific part of a DNA that encodes G α and modifying the amino acid sequence thereof. Accordingly, this system can be used similarly for screening for a ligand. Such technique can be 25 performed according to the method described in, for example, *Mol. Pharmacol.*, 57, 890-898 (2000) and *Biochemistry*, 37, 8253-8261 (1998).

For such fused protein (or mutant G α) expression cell, too, any form of intact cell, cell homogenate and membrane 30 fraction can be appropriately selected and used according to the screening method to be employed.

In another embodiment of the present invention, as a screening system containing, as constituent elements, a

lipid bilayer membrane containing GPRC5D protein or an equivalent thereof, and $\text{G}\alpha$ polypeptide, one obtained by re-constituting purified GPRC5D protein or an equivalent thereof with $\text{G}\alpha$ polypeptide, or a purified fused protein of 5 the receptor with $\text{G}\alpha$, in an artificial lipid bilayer membrane can be used. The GPRC5D protein or an equivalent thereof can be purified by affinity chromatography with the use of anti-GPRC5D antibody and the like from membrane fraction obtained from cerebral nerve tissue and the like including 10 hypothalamus of human or mouse, or other mammals.

Alternatively, the receptor can be purified by affinity chromatography using anti-GPRC5D antibody, His-tag, GST-tag and the like, from a recombinant cell into which an expression vector containing a DNA encoding GPRC5D protein or an 15 equivalent thereof has been introduced. Similarly, a fused protein of the receptor and $\text{G}\alpha$ can be also purified by affinity chromatography using anti-GPRC5D antibody, His-tag, GST-tag and the like, from a recombinant cell into which an expression vector containing a DNA encoding the fused protein has been 20 introduced.

As a lipid composing an artificial lipid bilayer membrane, phosphatidyl choline (PC), phosphatidyl serine (PS), cholesterol (Ch), phosphatidyl inositol (PI), phosphatidyl ethanolamine (PE) and the like can be mentioned. 25 A mixture of one or more kinds thereof mixed at a suitable ratio is preferably used.

For example, an artificial lipid bilayer membrane (proteoliposome) incorporating a receptor and $\text{G}\alpha$ or a receptor- $\text{G}\alpha$ fused protein can be prepared by the following 30 methods. First, a suitable amount of a mixed lipid chloroform solution of PC:PI:Ch=12:12:1 is separated in a glass tube, chloroform is evaporated in a nitrogen gas vapor to dry the lipid in the form of a film, a suitable buffer

is added to suspend the lipid, which is uniformly dispersed by ultrasonication, a buffer containing a surfactant such as sodium cholate and the like is further added to completely suspend the lipid. Thereto is added a suitable amount of 5 purified receptor and G α , or a receptor-G α fused protein, and after incubation for about 20-30 min while sometimes stirring in ice water, dialyzed against a suitable buffer, centrifuged at about 100,000xg for 30-60 min and the precipitation is recovered to give a desired proteoliposome.

10 A substance having a therapeutic activity against cibophobia or a lifestyle-related disease, which is selected by a screening system or a screening method as mentioned above can be prepared into a therapeutic agent for cibophobia or a lifestyle-related disease by combining any 15 pharmaceutically acceptable carrier.

Accordingly, the present invention provides a therapeutic agent for cibophobia, which comprises as an active ingredient an antagonist or an inverse agonist to GPRC5D, which is selected by the screening method of the 20 present invention. The present invention also provides a therapeutic agent for a lifestyle-related disease, which comprises as an active ingredient a physiological ligand or agonist to GPRC5D, which is selected by the screening method of the present invention.

25 The pharmaceutically acceptable carrier is exemplified by, but not limited to, excipients such as sucrose, starch, mannit, sorbit, lactose, glucose, cellulose, talc, calcium phosphate, calcium carbonate and the like, binders such as cellulose, methylcellulose, hydroxypropylcellulose, 30 polypropylpyrrolidone, gelatine, gum arabic, polyethylene glycol, sucrose, starch and the like, disintegrating agents such as starch, carboxymethyl cellulose, hydroxypropyl starch, sodium-glycol-starch, sodium hydrogen carbonate,

calcium phosphate, calcium citrate and the like, lubricants such as magnesium stearate, aerosil, talc, sodium lauryl sulfate and the like, aromatics such as citric acid, menthol, glycyl lysine ammonium salt, glycine, orange powder and the like, preservatives such as sodium benzoate, sodium bisulfite, methylparaben, propylparaben and the like, stabilizers such as citric acid, sodium citrate, acetic acid and the like, suspending agents such as methylcellulose, polyvinylpyrrolidone, aluminum stearate and the like, dispersing agents such as surfactant and the like, diluents such as water, physiological saline, orange juice and the like, base wax such as cacao butter, polyethylene glycol, refined kerosene and the like, and the like.

A preparation which is suitable for oral administration is, for example, a liquid comprising an effective amount of a ligand dissolved in a diluent such as water, physiological saline and orange juice, a capsule, sachet or tablet comprising an effective amount of a ligand as a solid or granules, a suspension comprising an effective amount of a ligand in a suitable dispersion medium, an emulsion comprising a solution of an effective amount of a ligand dispersed and emulsified in a suitable dispersion medium and the like.

A preparation preferable for parenteral administration (e.g., subcutaneous injection, intramuscular injection, topical injection, intraperitoneal administration and the like) includes, for example, an aqueous or non-aqueous isotonic sterile injection which may contain antioxidant, buffer, bacteriostatic agent, isotonicity agent and the like. It may be an aqueous or non-aqueous sterile suspension which may contain suspension, solubilizer, thickener, stabilizer, preservative and the like. When the administration method is topical injection near the hypothalamus, an injection

containing ligand as an active ingredient dissolved or suspended in an artificial cerebrospinal fluid is preferable. Alternatively, it can be formulated into a sustained release preparation using a biocompatible material such as collagen 5 and the like. Since pluronic gel gelates at body temperature and becomes a liquid at a lower temperature, long duration can be afforded by topically injecting the ligand along with pluronic gel to allow for gelation around the target tissue. The ligand preparation can be sealed in a container in a unit 10 dose or plural doses like an ampoule or vial. It is also possible to lyophilize a ligand and a pharmaceutically acceptable carrier and preserve them in a state that only requires dissolving or suspending in a suitable sterile vehicle immediately before use.

15 While the dose of the ligand preparation of the present invention varies depending on ligand activity (full agonist or partial agonist, or an antagonist or inverse agonist), degree of seriousness of the disease, the animal species to be the administration subject, drug acceptability, body 20 weight and age of the administration subject, and the like, it is generally about 0.0008 - about 2.5 mg/kg, preferably about 0.008 - about 0.025 mg/kg, a day for an adult in the amount of the ligand.

The present invention is explained in more detail by 25 referring to Examples, which are mere exemplification and not to be construed as limitative. Unless particularly specified, the following examples were performed according to the methods described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory 30 Press, New York (1989), Current Protocols in Protein Science (ed. Coligan, J. E. et al.), John Wiley and Sons, Inc. and the like.

Reference example 1: Analysis of expression distribution of GPRC5D gene by RT-PCR

The hypothalamus from db/db mouse (male, SPF grade, 7-week-old at the time of purchase, 10-week-old at the time 5 of organ sampling, CLEA JAPAN, INC.) was homogenized in Trizol (Gibco), and total RNA was obtained. cDNA was synthesized using RNA PCR Kit (AMV) Ver2.1. (Takara) and the total RNA (1 µg) as a template. PCR was performed using the obtained cDNA from hypothalamus as a template and the following mouse 10 GPRC5D (mGprc5d) specific primer. As a Taq polymerase, one from Perkin Elmer, Inc. was used.

5'-ACC TCT TCT GTG ACA ACG AG-3' (SEQ ID NO: 5)

5'-GGA AGA GGA CAT AGA TCA GG-3' (SEQ ID NO: 6)

As a result, expression of GPRC5D gene in the hypothalamus 15 of db/db mouse was clearly shown.

Reference example 2: Analysis of expression distribution of GPRC5D gene by *in situ* hybridization

After perfusion fixation with 10% neutral buffered 20 formalin, the brain was taken from mouse (CD-1(ICR)) and after fixation with 10% neutral buffered formalin, the block was prepared by paraffin embedding. The paraffin block was sliced in a 7µm-thick coronal section (in the vicinity of Interaural 2.34 mm, Bregma-1.46 mm), and used as a section 25 for ISH staining.

In addition, digoxigenin-labeled RNA probe was prepared by an In vitro transcription method with T3 and T7RNA polymerase. Specifically, antisense RNA probe corresponding to the region of 234th - 624th of SEQ ID NO: 3 30 was prepared using DIG RNA Labeling Mix (Roche).

Next, using mouse brain paraffin section and probe prepared above, ISH staining was performed by a conventional method. Using anti-digoxigenin antibody labeled with

alkaline phosphatase as antibody and NBT/BCIP as coloring substrate, nuclear staining with Kernechtrot was performed after staining.

As a result of staining using a GPRC5D antisense probe, expression in nucleus paraventricularis and hypothalamus ventromedial nucleus was found. The nucleus paraventricularis and hypothalamus ventromedial nucleus are the regions where the feeding center exists. Thus, GPRC5D was suggested to be a factor involved in the feeding and energy metabolism in the feeding center.

Example 1: Action of GPRC5D clone antisense DNA on obese model mouse

(1) Experimental materials

Reagent: As a GPRC5D clone antisense, a 25 mer thiolized antisense DNA was used, which corresponded to the vicinity of initiation codon of the gene. The base sequences of the antisense DNA are shown in the following.

5'-TCA TAC ATG GTG ACT TAT AGG TAG A-3' (SEQ ID NO: 7)

As a control DNA, thiolized oligo DNA having the following sequence was used.

5'-CCT CTT ACC TCA GTT ACA ATT TAT A-3' (SEQ ID NO: 8)

This sequence is complimentary to the sequence produced by the mutation which causes a splicing abnormality at position 705 in pre-mRNA of erythrocyte β -globin in hemoglobinopathy thalassemia. It is assumed that the oligosaccharide has no specific target region or biological activities in normal cells and corrects the splicing abnormality only when acted on erythrocyte of thalassemia, thereby producing normal β -globin coding mRNA. The synthesis, thiolation and HPLC purification of these oligo DNAs were committed to Nihon Bio Service Co., Ltd.

For other reagents, commercially available special

grade reagents were used.

Experimental animal: C57BL db/db (hereinafter, referred as obese mouse) (SPF grade) were purchased from CLEA JAPAN, INC., and after preliminary breeding, the obese mice were used for 5 the test at the age of 10 weeks.

Breeding environment: The mice were bred in a room controlled to a temperature of not lower than 20°C and not higher than 26°C, relative humidity of not less than 30% and not more than 70%, lighting cycle of 8:00-20:00 lighting and 20:00-8:00 10 lights-out. During breeding, the mice were allowed a free access to a solid feed (CE-2, CLEA JAPAN) and sterile distilled water.

(2) Preparation of administration liquid

As an antisense DNA administration liquid and a control 15 DNA administration liquid, an artificial cerebrospinal fluid (0.166 g/L CaCl₂, 7.014 g/L NaCl, 0.298 g/L KCl, 0.203 g/L MgCl₂·6H₂O, 2.10 g/L NaHCO₃) containing 7.5 µg/µl of antisense DNA was prepared.

(3) Administration of antisense DNA in cerebral ventricle

20 The obese mice were divided into two groups, and after fasting overnight, an antisense DNA administration liquid was given at 4 µl/mouse (30 µg/mouse for antisense DNA) to one group and a control DNA administration liquid (4 µl/mouse) was administered into the lateral ventricle of the other group, 25 simultaneously with the lighting at 8:00 a.m..

(4) Effect of antisense DNA administration on food consumption and blood glucose level of mice

Feeding of mice was resumed immediately after 30 administration, and food consumption was calculated every 12 hours up to 48 hours after administration. The effect of the antisense DNA on food consumption of obese mice is shown in Fig. 1. In Fig.1, black columns show average food consumption at every 12 hours after administration to control

DNA administration group and white columns show that of antisense DNA administration group (error bar is standard deviation, control DNA administration groups n=4, antisense DNA administration groups n=4). There was no big change in 5 the amount of food consumption up to 12 hours after administration. As the time advances, however, administration of the antisense DNA of GPRC5D clone showed an effect of increased food consumption in obese mice.

Additionally, blood glucose level was measured before 10 administration and every 24 hours up to 48 hours after administration. The effect of antisense DNA on blood glucose level of obese mice is shown in Fig. 2. In Fig.2, black columns show average blood glucose level at every 24 hours after administration of control DNA administration groups 15 and white columns show that of antisense DNA administration groups (error bar is standard deviation, control DNA administration groups n=4, antisense DNA administration groups n=4). There was no big change in the blood glucose level up to 24 hours after administration. At 48 hours after 20 administration, however, administration of the antisense DNA of GPRC5D clone showed an effect of increased blood glucose level in obese mice.

From the above-mentioned results, the possibility was suggested that GPRC5D clone is a factor involved in feeding 25 behavior and glucose metabolism.

Example 2: Analysis of variation in hypothalamus gene expression in satiation and fasting states

Hypothalamus was taken from db/db mouse (male, SPF grade, 30 7-week-old at the time of purchase, 11-week-old at the time of organ sampling, CLEA JAPAN, INC.; hereinafter to be referred to as obese mouse) and C57BL/6N mouse (male, SPF grade, 6

-week-old at the time of purchase, 11-week-old at the time of organ sampling, Charles River, Japan, Inc.; hereinafter to be referred to as normal mouse) in satiation state or 24 hour fasting state, homogenated in Trizol (Gibco) and total RNA was obtained. Reverse transcription reaction was performed using 1 µg of total RNA and SYBR Green RT-PCR Reagent (Roche). Subsequently, each sample after reverse transcription reaction and GPRC5D clone specific primer (see below) were mixed, PCR reaction was performed using ABI7700 sequence detector (PE biosystems) and variation in the expression of gene of GPRC5D clone was analyzed. In addition, GPRC5D clone expression was calibrated with GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and analyzed as relative values.

15 •GPRC5D

forward primer:

5'-GGA GTA TCT CAT CCC ATC AGC TAC A-3' (SEQ ID NO: 9)

reverse primer:

5'-CAC TCT TCC AGG TTG TCT CTA TGC-3' (SEQ ID NO: 10)

20 •GAPDH

forward primer:

5'-CAA GAG AGG CCC TAT CCC AAC T-3' (SEQ ID NO: 11)

reverse primer:

5'-CTA GGC CCC TCC TGT TAT TAT GG-3' (SEQ ID NO: 12)

25 Variation in expression of GPRC5D gene in the satiation/fasting state is shown in Fig. 3. In Fig. 3, black columns show average variation in the expression of GPRC5D gene in satiation state, white columns show that in the expression of GPRC5D gene in the fasting state (error bar shows standard deviation, n=3 in each groups). There was found no big variation in normal mouse. On the contrary, promoted expression of GPRC5D gene was found in obese mouse in the fasting state. The promoted expression of GPRC5D

clone in obese mouse in the fasting state and promoted food consumption and increased blood glucose level due to the administration of antisense suggested the possibility of GPRC5D clone having a food consumption suppressive action.

- 5 From the above findings, it has been clarified that GPRC5D clone is involved in the regulation of food consumption and glucose metabolism, and that promotion of the action of GPRC5D clone provides a more effective therapeutic effect on lifestyle-related diseases.

10

Example 3: Establishment of GPRC5D stable expression cell line

(1) Construction of transgene vector

The coding region of GPRC5D clone is amplified by PCR 15 method using KOD-Plus (TOYOBO). The amplified gene fragment is inserted into pCDNA3.1 (Invitrogen) and a GPRC5D expression vector is constructed.

(2) Establishment of cell line

CHO cell lines expressing three types of chimeric G 20 proteins (Gq, Gqi5, Gqs5) are seeded in 10 cm² culture dishes and cultured in a D-MEM medium (Gibco) containing 10% FBS (Gibco) until 60-70% confluent. Then, the cells are transferred to a serum-free medium, complexed with the GPRC5D expression vector constructed in the above (1) and 25 Lipofectamine-Plus (Gibco), and added to the medium. After incubation for 5 hours, the medium is changed to D-MEM medium containing 10% FBS and the cells are further cultured for 8 hours. Then cells are detached from the petri dish with trypsin-EDTA, suspended in D-MEM medium containing 500 µg/ml 30 G418 and 10% FBS and seeded in 10 cm² petri dishes. Few days later, formed colonies are isolated and named as GPRC5D stable expression cell lines (Yq, Yqi5, Yqs5).

Example 4: Screening for receptor agonist

(1) Preparation of cells

GPRC5D stable expression cell lines (Yq , Yqi5 , Yqs5) are seeded in 96-well culture dishes and cultured in a D-MEM medium (Gibco) containing 10% FBS (Gibco) until 60-70% confluent. They are used as expression cells.

(2) Addition of reagents

The medium of expression cells is changed to serum free D-MEM medium one day before use. On assessment day, each compound (2.5 mM DMSO solution) is diluted to desired concentrations with D-MEM medium. Medium in the culture dishes is removed, and the diluted test compound, 4 μM Fluo3-AM (Teflab.) and 2.5 mM probenecid are added and it is cultured at 37°C for 60 min. A sample treated in the same way except that the test compound is not added is prepared for comparison.

(3) Measurement of intracellular calcium concentrations by FLIPR method

The cells treated as mentioned above are washed with ice-cooled PBS, and suspended in Thyrode's medium (containing 2.5 mM probenecid and 1% gelatin). Absorbance of culture dish at 488 nM and 540 nM is quantified by FLIPR (Molecular Devices).

25 **Example 5: Construction of plasmid for expression of GPRC5D-G α fused protein**

As three kinds of representative α subunits considered to almost cover all GPCR signaling, G α 16 was selected from the G q family, G α i2 was selected from the Gi family and G α S2 was selected from the Gs family. All these coding regions were PCR cloned into expression vector pcDNA3.1(+). A restriction enzyme EcoRV cleavage site and a sequence containing 6 \times His tags were added just before each G α coding

region by PCR, whereby plasmids pcHISG α 16, pcHISG α i2 and pcHISG α S2 capable of easy fusion with GPCR gene on the 5' side of each G α protein were prepared.

That is, human spleen cDNA (Clontech) was diluted 20 times, 1 μ l thereof was used as a template for amplification. As the enzyme, employed was KODplus (TOYOBO). However, the Mg $^{2+}$ concentration was 1 mM. For amplification reaction, 20 μ l of a reaction solution was used, which was a reaction buffer attached with KODplus. To amplify G α 16 cDNA, primer GNA15F1 (SEQ ID NO: 13) and GNA15R1 (SEQ ID NO: 14) were used; to amplify G α i2 cDNA, primer GNAi2F1 (SEQ ID NO: 15) and GNAi2R1 (SEQ ID NO: 16) were used; and to amplify G α S2 cDNA, primer GS2F1 (SEQ ID NO: 17) and GS2R1 (SEQ ID NO: 18) were used. For amplification, GeneAmp PCR System 9600 (Applied Biosystems) was used and amplification was conducted under the conditions of 94°C, 2 min \rightarrow (94°C, 15 sec \rightarrow 68°C, 120 sec) \times 40 cycles to give a PCR product having an object size. The terminal of each PCR product was phosphorylated, separated and purified by 0.8% agarose gel electrophoresis, cleaved with restriction enzyme EcoRV, ligated with plasmid pcDNA3.1(+) (Invitrogen) treated with CIAP and used to transform *Escherichia coli* DH5 α . Plasmids were purified from the transformant line, and upon confirmation that the restriction enzyme digestion pattern and inserted base sequence were objective ones, the obtained G protein expression plasmids were named pcG α 16, pcG α i2 and pcG α S2, respectively.

To fuse GPRC5D via HIS tag on N terminal of each G protein, HIS tag sequence was ligated immediately before initiation codon ATG of each G protein, and a restriction enzyme EcoRV recognition site was further added. That is, PCR was conducted using 10 ng each of pcG α 16, pcG α i2 and pcG α S2 as a template, a primer (GNA15ATG; SEQ ID NO: 19,

GNAi2ATG; SEQ ID NO: 20 or GS2ATG; SEQ ID NO: 21) and a primer (pcDNARV; SEQ ID NO: 22) of vector. Amplification was conducted under the conditions of 94°C, 2 min → (94°C, 15 sec → 58°C, 30 sec → 68°C, 60 sec) × 20 cycles to give a 5 PCR product having the object size. Then HIS2 linker (SEQ ID NO: 23) and HIS2 linker(R) (SEQ ID NO: 24) were annealed, and after phosphorylation of the terminal, ligated with each PCR product. These were digested with restriction enzymes EcoRV and XhoI, and the object DNA fragments were separated 10 and purified by 0.8% agarose gel electrophoresis. The recovered DNA fragments were ligated with expression plasmid pcDNA3.1(+) digested with EcoRV and XhoI, and used to transform *Escherichia coli* DH5α. Plasmids were purified from transformant line, and upon confirmation that the 15 restriction enzyme digestion pattern and inserted base sequence were object ones, the obtained plasmids were named pCHISGα16, pCHISGαi2 and pCHISGαS2, respectively.

Example 6: Construction of GPRC5D-Gα fused protein

20 **expression plasmid**

(1) cloning of GPRC5D

PCR cloning of GPRC5D gene was performed with human testis derived cDNA library (clontech) and the following primers.

25 5'-GGAGAAGGGCATCAGAAAAC-3' (SEQ ID NO: 25)

5'-TTATACTCCTGCATCTTGC-3' (SEQ ID NO: 26)

The obtained fragment was subjected to TA-cloning with pT7Blue vector (Novagen). The plasmid was prepared from the obtained clone, and analyzed for the base sequence. As a 30 result, GPRC5D gene having a sequence identical to the sequences of published references was obtained. The obtained plasmid was named as pT7GPRC5D.

(2) construction of GPRC5D expression vector

Construction of expression vector was carried out using GATEWAY system of Invitrogen Corp. and in accordance with the vendor's instructions. First, expression vectors were converted to destination vectors. pcHISGαS2, pcHISGαi2 and 5 pcHISGα16 prepared in example 5 were each digested with EcoRV then GATEWAY frame B (Invitrogen) was inserted. DB3.1 competent cells (Invitrogen) after transformation were selected with chloramphenicol, the obtained clones were further selected by digesting with restriction enzymes to 10 give desired clones having GATEWAY frame B insert in the right direction. These were named as pcHisGαS2-DEST, pcHisGαi2-DEST and pcHisGα16-DEST, respectively. In addition, a destination vector having the same CMV promoter and bGH terminator was used as a non-fused gene expression 15 vector for control. Specifically, pcDNA3.1mycHisA was digested with Hind III-Xba I and then blunted, and GATEWAY frame C.1 (Invitrogen) was inserted. Then DB3.1 competent cells (Invitrogen) after transformation were selected with chloramphenicol, the obtained clones were further selected 20 by digesting with restriction enzymes, and a clone having GATEWAY frame C.1 insert in the right direction was obtained, which was named as pcDNA3.1-DEST.

Next, using pT7GPRC5D as a template and the following primers, ORF (R-type) having up to intact stop codon, and 25 ORF (F-type) with only intact stop codon deleted were amplified by PCR. After electrophoresis and purification by cutting out from the gel, these ORFs were subjected to BP clonase (Invitrogen) reaction to be newly carried on donor vector pDONR201.

30 atGPRC5DM (forward primer):

5'-GGGGACAAGTTGTACAAAAAAGCAGGCTCCACCAatgtacaaggactgcacg
agtcc-3' (SEQ ID NO: 27)

atGPRC5DR (reverse primer for R-type):

5'-GGGGACCACTTGTACAAGAAAGCTGGGTCA~~tata~~actcctc~~tgc~~atcttg
tg-3' (SEQ ID NO: 28)

atGPRC5DF (reverse primer for F-type):

5'-GGGGACCACTTGTACAAGAAAGCTGGGT~~C~~actcctc~~tgc~~atcttgctg-3
5 ' (SEQ ID NO: 29)

By analysis of the base sequence of the obtained clones, desired clones were each confirmed to have been obtained. The obtained clones were named as pENTR/GPRC5D/R (R-type entry clone) and pENTR/GPRC5D/F (F-type entry clone), 10 respectively.

By LR clonase reaction of these, pENTR/GPRC5D/F was crossed over 3 kinds of G α fused destination vectors (pcHisG α S2-DEST, pcHisG α i2-DEST and pcHisG α 16-DEST) and pENTR/GPRC5D/R was crossed over pcDNA3.1-DEST. In these 15 obtained clones, plasmids were prepared on a small scale and the desired clone was selected by restriction enzyme treatment. The obtained plasmids were named as pcC5D/His/G α S2, pcC5D/His/G α i2, pcC5D/His/G α 16 and pcC5D, respectively. Further, using Qiagen Maxi Kit, mass 20 preparation and purification of plasmid were done from 100 ml of culture fluid with the aim of introduction into cell lines.

Example 7: Confirmation of constitutive activation by 25 expression of GPRC5D-G α fused protein

To examine whether constitutive activation occurs by cellular expression a protein with G α protein fused at the c-terminal of GPRC5D, the following experiment was carried out.

30 1 μ g of DNA for four kinds of plasmid vectors (pcC5D, pcC5D/His/G α S2, pcC5D/His/G α i2 and pcC5D/His/G α 16) constructed in Example 6, and pcDNA3.1(+) (Invitrogen: Cat. No. V790-20) were diluted with 125 μ l of OPTI-MEM I medium

(Invitrogen: Cat. No. 31985-062) (solution A). 2.5 μ l of Lipofectamine 2000 Reagent (Invitrogen: Cat. No. 1168-027) which is a transfection reagent, were diluted with 125 μ l of OPTI-MEM I medium and stood for 5 minutes (solution B).

5 Solution A and solution B were mixed and incubated for 20 minutes, and then added at 50 μ g/well (triplicate) to Chinese Hamster Ovary cells (CHO-K1 cells, ATCC No. CCL-61) plated 3×10^4 cells/well the day before. After culture at 37°C for 4 hours under condition of 5% CO₂, the medium was changed to

10 F12 medium (Invitrogen: Cat. No. 11765-054) containing 10% FCS at 100 μ l/well and further cultured for about 12 hours. Intracellular cAMP levels of these cells were measured using HitHunter™ EFC Cyclic AMP Chemiluminescens Assay Kit (Applied Biosystem: Cat. No. DRX-0027), cAMP measurement kit,

15 and in accordance with the attached protocol (Fig. 4). As a result, cAMP levels were confirmed to have been specifically increased by fusing GPRC5D with G α S2, suggesting that GPRC5D can be constitutively activated by fusing with G α S2. Therefore, the GPRC5D was strongly suggested to be a GPCR

20 that is coupled with G α S.

Sequence Listing Free Text

SEQ ID NO: 5: Oligonucleotide designed to function as a primer to amplify mRNA of GPRC5D.

25 SEQ ID NO: 6: Oligonucleotide designed to function as a primer to amplify mRNA of GPRC5D.

SEQ ID NO: 7: Oligonucleotide designed to function as an antisense DNA inhibiting expression of GPRC5D.

30 SEQ ID NO: 8: Oligonucleotide designed to function as an antisense DNA for sequence resulted from mutation causing abnormal splicing at position 705 of β -globin pre-mRNA in thalassemia.

SEQ ID NO: 9: Oligonucleotide designed to function as a

primer to amplify mRNA of GPRC5D.

SEQ ID NO: 10: Oligonucleotide designed to function as a primer to amplify mRNA of GPRC5D.

SEQ ID NO: 11: Oligonucleotide designed to function as a 5 primer to amplify mRNA of GAPDH.

SEQ ID NO: 12: Oligonucleotide designed to function as a primer to amplify mRNA of GAPDH.

SEQ ID NO: 13: Oligonucleotide designed to function as a sense 10 primer to amplify human G protein α 16 cDNA fragment containing full length ORF.

SEQ ID NO: 14: Oligonucleotide designed to function as an antisense primer to amplify human G protein α 16 cDNA fragment containing full length ORF.

SEQ ID NO: 15: Oligonucleotide designed to function as a 15 sense primer to amplify human G protein α i2 cDNA fragment containing full length ORF.

SEQ ID NO: 16: Oligonucleotide designed to function as an antisense primer to amplify human G protein α i2 cDNA fragment containing full length ORF.

20 SEQ ID NO: 17: Oligonucleotide designed to function as a sense primer to amplify human G protein α S2 cDNA fragment containing full length ORF.

SEQ ID NO: 18: Oligonucleotide designed to function as an 25 antisense primer to amplify human G protein α S2 cDNA fragment containing full length ORF.

SEQ ID NO: 19: Oligonucleotide designed to function as a sense primer to amplify human G protein α 16 cDNA fragment from the initiation codon.

30 SEQ ID NO: 20: Oligonucleotide designed to function as a sense primer to amplify human G protein α i2 cDNA fragment from the initiation codon.

SEQ ID NO: 21: Oligonucleotide designed to function as a sense primer to amplify human G protein α S2 cDNA fragment from

the initiation codon.

SEQ ID NO: 22: Oligonucleotide designed to function as an antisense primer to amplify multicloning sites of plasmid pcDNA3.1(+) .

5 SEQ ID NO: 23: Sense chain oligonucleotide designed to construct a linker containing a nucleotide sequence encoding a 6xHis tag peptide sequence.

SEQ ID NO: 24: Antisense chain oligonucleotide designed to construct a linker containing a nucleotide sequence encoding

10 6xHis tag peptide sequence.

SEQ ID NO: 25: Oligonucleotide designed to function as a sense primer to amplify cDNA of GPRC5D.

SEQ ID NO: 26: Oligonucleotide designed to function as an antisense primer to amplify cDNA of GPRC5D.

15 SEQ ID NO: 27: Oligonucleotide designed to function as a sense primer to amplify ORF of cDNA of GPRC5D.

SEQ ID NO: 28: Oligonucleotide designed to function as an antisense primer to amplify ORF(R-type) of cDNA of GPRC5D.

SEQ ID NO: 29: Oligonucleotide designed to function as an 20 antisense primer to amplify ORF(F-type) of cDNA of GPRC5D.

Industrial Applicability

Since GPRC5D is a GPCR involved in feeding behavior, the pharmaceutical composition of the present invention

25 containing, as an active ingredient, a substance that enhances or suppresses expression or function of GPRC5D can regulate food intake to a desired level and is expected to afford a therapeutic effect on lifestyle-related diseases caused by overeating, such as diabetes, obesity,

30 hyperlipidemia and the like, or cibophobia. According to the screening system and screening method of the present invention, moreover, a ligand for GPRC5D can be easily and rapidly screened for and they are useful for the development

of a new drug targeting GPRC5D, search for a disease marker and establishment of a diagnostic method using the disease marker.

5 While the present invention has been described with an emphasis on preferred embodiments, it will be obvious to those of ordinary skilled in the art that variations of the preferred embodiments may be used. It is intended that the invention may be practiced otherwise than as specifically described
10 herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

This application is based on a patent application No.
15 397523/2001 filed in Japan, the contents of which are hereby incorporated by reference. The references cited herein, including patents and patent applications, are hereby incorporated in their entireties by reference, to the extent that they have been disclosed herein.

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